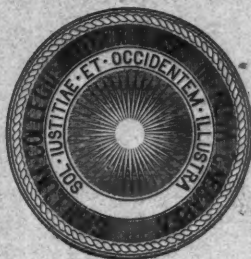


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CULTURAL STUDIES OF SPECIES OF ACTINOMYCES

SELMAN A. WAKSMAN

Department of Soil Bacteriology, New Jersey Agricultural Experiment Station

Received for publication July 15, 1919

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INTRODUCTION

The actinomycetes form a large and important group of microorganisms, both in numbers and activities. They have been isolated from wounds, air, water and foodstuffs, but chiefly from the soil. Their functions are as variable as those of any other large group of living forms, so that we cannot point to any particular activity as their probable rôle in nature. As any other large group of forms of life, they are extremely variable: both their morphology and physiology vary with the environmental conditions, and any classification which is based upon the cultural or morphological studies on one medium, is artificial and will not accomplish any far-reaching results in establishing the identity of the species.

Many actinomycetes, chiefly the pathogenic forms (animal), were studied on complex organic media, upon which they produced an abundant, but uncharacteristic growth. The basis for study was necessarily the physiological activities of the organisms, but these, as will be pointed out soon, cannot be used as a basis for classification, when only a few strains are available. The characteristic morphology of the actinomycetes is brought out only on simple

* Colored plates to illustrate this article were prepared by the author, but on account of lack of funds it was necessary to omit them. It is hoped they will be published at some later date.

synthetic media, particularly when the nitrogen and carbon sources do not allow any abundant growth of the organisms, so that they do not form much of a substratum growth (which is uncharacteristic in most cases) but are found to produce an aerial mycelium, characteristic for the different organisms, and thus allow a detailed study of their morphology.

Slight differences in physiology or cultural characters have often served for the differentiation of species. When a large number of actinomycetes are studied, as in the case of the present investigation, where over 300 isolations were made, and numerous cultures obtained from other sources, these studies lasting for over four years, it will be found that differences observed are often quantitative rather than qualitative in nature. Several strains belonging to one group may show at one time differences on one medium but not on others, and, on repeated cultivation, this difference may disappear and another difference may arise. These minor changes depend on the slight variation in the composition of the medium, amount of inoculum added, form of inoculum (spore material, surface or subsurface growth), age of culture, length of time since it was isolated from its natural habitat, etc.

All the cultures should be divided into groups, the representatives of which have common morphological, physiological and cultural characters. These species groups may show slight variations within the groups, when several representatives are compared, but all of them possess in common the main distinguishing characters of the species, and are distinctly different from any other species group. Quite often cultures are obtained which form transition steps between two different species. In that case they are either classed with the species to which they approach nearest, or left as transition forms, or, if distinct enough from the two nearest species, placed into species by themselves. Another difficulty in establishing species is encountered in attempting to fix the amount of variation necessary to constitute a new species. The difference of various strains in the same groups is chiefly that of degree only.

Certain cultural characters may either be lost or gained on continued cultivation (such as pigment production on gelatin or rapidity of liquefaction, etc.) and it is, therefore, advisable to describe species of actinomycetes soon after their isolation from their natural habitat and after prolonged cultivation upon artificial culture media.

HISTORICAL

No attempt will be made here to review the numerous investigations published on this group of organisms. Attention will be called here only to the more important works which contributed largely to the advance of our knowledge on this group of organisms, where also a complete bibliography of the previous investigations can be found.

Cohn (8) first described (1875) an Actinomyces under the name of *Streptothrix Foesteri*. But this name (*Streptothrix*) is untenable, since it was pre-

empted by Corda for a genus of true fungi. The next species *Actinomyces bovis* was described by Harz (21) in 1877, and the name used by this investigator has good claims to priority and will therefore be used throughout this work; Harz placed *A. bovis* among the Hyphomycetes. Trevisan (36) applied, in 1889, the term *Nocardia* to a saprophytic form. This term could be used, if the organisms belonging to this group could be divided into 2 subgroups, parasitic and saprophytic, as attempted by the committee of the American Bacteriological Society on Generic Classification, but this subdivision cannot hold, since no distinctive differences could be found both culturally and morphologically, between these two groups and the mere fact that one species was isolated from the soil and another from a wound cannot justify their placing in two distinct genera.

Domec (12) gave a detailed description of the morphology of *A. bovis* (Harz) together with an accurate account of spore formation; he demonstrated that these are really the spores of a mold fungus not of a fission fungus.

Nadson (29) isolated several species of *Actinomyces* from the curative mud of the Slavian mineral waters in Russia and suggested that the genus should be placed in a group of fungi by itself.

Gedoeft (18) placed this group among the Fungi Imperfecti, in the genus *Discomyces* Rivolta (1889), with *Streptothrix* Cohn (1875), *Actinomyces* Harz (1877), *Nocardia* Trevisan (1889), *Oospora* Savagean and Rádais (1892) and *Discomyces* Blanchard (1900) as synonyms. Petruschky (33) classified the actinomycetes with the *Trichomycetes* of the hyphomycetes, recognizing 4 groups: *Actinomyces*, *Streptothrix*, *Cladothrix* and *Leptothrix*. A thorough discussion and criticism of these early attempts of classification can be found in the work of Lehmann and Neumann (24), Lachner-Sandoval (23) and those mentioned in the following pages. Musgrave, Clegg and Polk (28) in 1908 gave a detailed study of the pathogenic forms known and almost a complete bibliography.

Claypole (7) was among the first to call attention to the great variability in morphology and cultural characters of this group of organisms, which is the cause of confusion and diverse opinions and practices. These organisms change their appearance on different culture media. She suggested that this group, with their variable morphology and close relationships, should be looked upon as representing the ancestral type of both the higher fungi and the true bacteria.

The production of a pigment by the actinomycetes has early attracted the attention of the investigators as a basis for classification. Gasperini (17) thought to have definitely established the identity of *A. chromogenus*, which is supposed to produce a brown pigment in gelatin or peptone agar, which rapidly diffuses through the medium. Krainsky (22) and later Waksman and Curtis (44) and Conn (10) have shown that the pigment production is an insufficient basis for the characterization of the species. A very large number of strains of this group of organisms can produce a brown (of different shades)

pigment on gelatin, potato and peptone agar; this property, as was pointed out above, is not a constant characteristic for all types. Sanfelice (35), recognizing the great variability of these organisms, suggested a division into groups, but he used as a basis for classification the color production upon complex organic media; had he used synthetic media, standard in composition, this division might have been of some value, but the pigment production upon complex organic media is a very indefinite basis for any attempt to subdivide the organisms.

Krainsky (22) recognized this difficulty and was the first to suggest the use of simple synthetic media, standard in composition, for the study of this group. He described 18 species of saprophytic actinomycetes which are undoubtedly the most complete descriptions that appeared in all the literature; but even his work has not been sufficient enough that one would be able to identify with certainty any species that he has described.

The writer with Curtis (44) published a preliminary report on the actinomycetes in the soil, of which the following work, after a careful and continuous study for 4 years, is a result. They attempted to classify the actinomycetes on the basis of liquefaction and pigment production in gelatin and spiral formation in aerial mycelium. This method was justly criticised by Conn (10), who however did not suggest anything more definite; if anything, his cultural studies, adding some information, cannot go toward classification even as far as the division into types producing macro and micro colonies, as suggested by Krainsky (22), or spiral formation and liquefaction and pigment production in gelatin, used by Waksman and Curtis (44). The latter stated that some species that they isolated from the soil were the same as those isolated by Krainsky (22), but since the comparison was based only on description of the latter's cultures, certain differences were found on further study; but several species, such as *A. viridochromogenus*, were found to correspond very closely with the descriptions of Krainsky on all media. A few cultures were either lost on transfer since the first description, or were found to fall into one group with others, with which they were properly placed.

Conn (10) mentioned three main types of soil actinomycetes and described one new species. Waksman and Curtis (45) demonstrated the wide occurrence of actinomycetes in soils under different conditions of climate, topography and cultivation. Certain species (or groups) were found to be very abundant in the soil and were isolated from soils located in different parts of the world, while others were isolated only once or twice. The writer (41, 43) recently published a series of investigations on the metabolism of the actinomycetes, and some of the data published there will be repeated here for the sake of completeness of the work.

Drechsler (13) has made the most complete morphological study that we have so far of the genus *Actinomyces*, and concluded that it should be classed with the Hyphomycetes, as a Mucedineous group with tendencies toward an erect Isarioid habit. If the bacteriologists that studied this group can be criticised

for paying little attention to the true morphology of the organisms, the botanists should be criticised, when studying the morphology of a large group of microorganisms like the actinomycetes, in entirely neglecting the effect of environmental conditions, particularly the composition of media.

It has been repeatedly pointed out (25, 43) that the composition of the culture medium affects the character of the colonies of bacteria and even fungi to such a degree as to make it necessary to have media of a standard chemical composition and to have an exact description of these media. Particularly such a variable group as the actinomycetes, which give growth distinctly different in many respects, with a mere change of the source of nitrogen or carbon, should be cultivated on media of exact chemical composition, especially when exact morphological characters are studied. The most excellent morphological studies of Drechsler have this one disadvantage—they have been carried out on media of variable composition (peptone glucose agar and potato agar), at an unknown temperature and for an unknown length of time. Any one of these factors will affect the biochemical and cultural characters, as well as the morphological.

NOMENCLATURE

The generic name

The systematic position of this whole group of microorganisms has been discussed before. All that we know about their structure and development would make us recognize them as true fungi—*Eumycetes* Brefeld. They produce a very fine, unicellular mycelium, well developed and abundantly branched. They reproduce by means of aerial spores, or so-called conidia, which have nothing in common with bacterial forms of reproduction, but resemble greatly those of many fungi. They are easily separated from the bacteria by their morphology as well as cultural characters; they are differentiated by the true branching, character of growth and type of conidia from the bacteria characterized by the formation of false branching, such as *Cladothrix* and those producing involution forms (*B. tuberculosis*).

They cannot be classified with the fungi *Oospora*, *Streptothrix*, or *Discomyces* for the obvious reason that those forms are multicellular. The term *Actinomyces* Harz is therefore the most valid, particularly since it characterizes the type of growth. The suggestion of Lechner-Sandoval (23), Lehmann and Neumann (24) and Drechsler (13) to classify the genus in an unqualified manner with the Hyphomycetes will meet certain objections. The looseness of the group Hyphomycetes, where botanists have piled together forms which could not be placed elsewhere, and the fact that these have a septated mycelium, will make the place for the actinomycetes improper, and it will not help much to advance the question of the proper classification. The author can only agree with Nadson (29), who stated yet in 1900 that the actinomycetes form a special group of fungi, to be classified separately, until new facts concerning the history of their development are found.

Nomenclature of the species

As a basis for the nomenclature of the species, the organisms described by the writer and Curtis (44) and pathogens obtained from known sources were taken. The well described cultures of Krainsky (22) could not be obtained and use had to be made only of his descriptions; the identifications in that respect may be questionable, particularly in certain cases. *A. pheochromogenus* was isolated by the writer from the soil, but was first described by Conn (10). *A. poolensis* was obtained from Dr. Taubehaus (37) and also isolated from the soil. A few new species are described so as to make the study of the group complete, although, as stated above, care was taken to exclude as many of the types which approached the described species as possible. Attention will be called to this fact in the process of the work. It cannot be contended that all these species are new to science, since it is possible that certain of them have already been described before. But the fact that all the old cultures are unavailable for examination, while this publication applies to definite material, with less probability for confusing the different species when studied in one laboratory, will justify this course.

This paper lays no claim to completeness as a monograph of the genus Actinomyces. As many authentic cultures were secured as possible and careful study was made of all the published descriptions. This paper represents cultural and biochemical studies continued for over four years at several institutions and includes only those species for which the data obtained abundantly justify the characterization. Most forms were isolated by the author from the soil, a few others particularly the pathogenic forms, were obtained from other sources, as will be pointed out in the description. In this work the author was assisted during 1915-1916 by Mr. R. E. Curtis at the New Jersey Agricultural Experiment Station, who deserves a great deal of credit for the cultivation of the organisms and some biochemical studies; the work was then continued by the author during 1916, 1917 and 1918 in the Department of Biochemistry, University of California and at the Cutter Biological Laboratories, Berkeley, Cal. The work was completed during the college year 1918-1919 at the New Jersey Station, where the writer was assisted by Mr. Jacob Joffe in carrying on and checking up some cultural and biochemical studies and by Mr. Willem Rudolfs in making microscopic studies of the morphological characters of the organisms. The author takes here the opportunity to express his sincere thanks to Mr. Curtis, Mr. Joffe and Mr. Rudolfs, to the institutions where the work was carried on, and to Dr. Charles Thom and Dr. H. J. Conn for reading the manuscript.

OCCURRENCE OF ACTINOMYCETES IN NATURE

Actinomycetes have been isolated from numerous sources: as animal and plant pathogens, air, water, milk, salt water lakes and soil. One reason for their wide occurrence is the ease with which they can adapt themselves to a

new environment. Certain forms will grow in salt water or ordinary tap water without the addition of any nutrients; this is the reason why certain investigators may be led to suspect that they can fix atmospheric nitrogen. Also, the spores are very light and can easily be carried in the air for a long time. The ease of adaptability can be seen from the fact that when 3 animal pathogens, *A. bovis*, *A. madurae* and *A. hominis* were inoculated upon sterile soil to which some nitrogenous organic material ($\frac{1}{2}$ per cent of dried blood) had been added, they made a good growth upon it and were subsequently reisolated from the soil. The ease with which they grow on organic media is due to their ability to decompose organic substances readily. For a complete study of the occurrence of the actinomycetes in nature, reference can be here made to the work of Musgrave and associates (28), where the pathogenic forms are studied, and to the studies of Krainsky (22), Waksman and Curtis (45) and Conn (10) for the occurrence of saprophytic forms.

MORPHOLOGICAL STUDIES

Very little morphological work (Neukirch (30)) has been done on this group of organisms before that of Drechsler (13); this was due to the minuteness of the typical characters of these organisms, but primarily to the fact that most media used allowed a rather uniform development. Nadson (29) pointed out as early as 1900 (work published in 1903 and apparently overlooked by subsequent investigators) some of the typical morphological characters of these organisms: hyphae are thin, colorless, cylindrical, 0.5μ to 0.7μ in diameter, the growing portions being only about 0.3μ , straight or forming spirals; hyphae branch abundantly, the branching being true, similar to that of fungi and distinctly different from the false bacterial branching (such as *Cladotrix dichotoma*); the mycelium is not septated. The contents of the young hyphae are found, under the microscope, to be pale; homogeneous granules appear in older cultures and in places the plasma breaks up into elongated portions, separated by more colorless intervals. The aerial hyphae break up into branching lines of spores, usually called aerial conidia, but more properly termed, by type of formation, oidia; these are short elliptical $0.75 \times 1.25 \mu$. Numerous involution forms are found in older cultures, both in substratum and aerial mycelium including the conidia. The hyphae are thin regularly cylindrical, turning wide in many places, forming club-like balloons; these swollen portions of the hyphae are often transformed into a series of spherical or elliptical ampules, united by thin channels, the end of the hyphae may develop into a club-like form; this, as well as those following, may be separated from the hyphae into a free spherical body; in other cases the hyphae, becoming thicker (1.25μ – 1.50μ), develop into screw-like forms or spirals, which may freely separate from the hyphae from which they originated; the clubs of the pathogenic *Actinomyces bovis*, although somewhat different in structure, belong to these involution forms.

A complete study of the work done by previous investigators, particularly that of Lechner-Sandoval (23) and Neukirch (30) is given in the paper by Drechsler (13), who made a thorough study of the morphology of this group of organisms, which, with certain limitations, as was pointed out above, is the most complete work done on this subject. In view of the fact that the work of Drechsler was carried on, to some extent, with the organisms reported by the author and also treated in the following pages of this paper, and since it was almost impossible at present to carry on any extensive morphological studies to such a degree as carried out by Drechsler, the morphology of the different species studied will not be taken up to any large extent in this paper. It may though not be out of place to give the summary of Drechsler's work (page 161).

1. The vegetative thallus of *Actinomyces* consists of a mycelium composed of profusely branching hyphae, the terminal growing portions of which are densely filled with protoplasm. Toward the center of the thallus the vacuoles increase in size and may be associated with the presence of metachromatic granules, the latter having in common nothing with bacterial endospores or "micrococci," for which they were mistaken by early observers.

2. The vegetative mycelium attains an extent incomparably greater than the branching figures recorded for bacteria of the acid-fast group, and the hyphae lack the uniformity in diameter generally characteristic of the *Schizomycetes*.

3. The aerial mycelium produced on suitable substrata by most species occurs usually in the form of a mat of discrete fructifications; but in other species these fructifications are frequently combined to form numerous and peculiar erect Isarioid sporodochia.

4. In any case each individual fructification represents a well characterized sporogenous apparatus, consisting of a sterile axial filament bearing branches in an open racemose or dense capitate arrangement. The primary branches may function directly as sporogenous hyphae, or may proliferate branches of the second and of higher orders, sporogenesis in the latter case being confined to the terminal elements, the hyphal portions below the points of attachment of branches remaining sterile.

5. Two tendencies in the development of fructifications are recognizable: one leading to an erect dendroidal type, in which successively proliferated fertile elements undergo processes of sporogenesis in continuous sequence; and the other leading to a prostrate racemose type, in which sporogenesis is delayed in the older branches until the younger branches have also attained their final extension. The majority of species show these tendencies combined in different ways.

6. The sporogenous hyphae of most species are coiled in peculiar spirals, sometimes resembling the spores of the hyphomycetous genus *Helicium*. These spirals exhibit pronounced specific characteristics in the number, diameter, and obliquity of their turns, and especially in the direction of rotation (whether dextrorose or sinistrorse).

7. Sporogenesis, where it can be followed, begins at the tips of the fertile branches and proceeds basipetally. In the larger number of species the process involves the insertion of septa which, in certain cases, are relatively very massive, and in others so thin as to be barely discernible. The disposition of these septa, while the delimited spores undergo maturation processes, varies with the species: (a) they may remain more or less unaltered; (b) they may suffer a median split, the two resulting halves being then separated as the result of the longitudinal contraction of the young spores, leaving alternate portions of hyphal walls completely evacuated; or (c) they may first become considerably constricted and subsequently converted into non-stainable isthmuses connecting the mature spores. The apparent absence of septa in the sporogenous hyphae of other forms is perhaps attributable to optical difficulties.

8. Granules are readily differentiated in the spores of many species which possess the staining properties and uniformity of size characteristic of nuclei; they generally occur singly, but in the larger spores of a few forms are often found occupying diagonally opposite positions.

9. As in the vegetative thallus, metachromatic granules occur in the aerial mycelium, being very rarely found in the spores or sporogenous hyphae, but becoming very abundant in degenerate sterile hyphae.

10. The older axial filaments of some species show marked distensions which, in extreme cases, result in figures simulating *Leptomitus*. These arise as local distensions at the points of attachment of the more extensive lateral sporogenous processes. Cuneate modifications of the sterile axial filaments below the origins of branches also occur.

11. Curious spherical structures appear regularly in some forms, both in the sterile axial hyphae, where they may contain either a median septum or a number of peripheral metachromatic granules, and in the sporogenous hyphae, where they are associated with the regularly spaced septa.

12. The spores germinate readily in suitable solutions, producing 1-4 germ tubes, the approximate number being more or less characteristic of the species.

CULTURAL AND BIOCHEMICAL STUDIES

The importance of using simple media and giving their exact chemical composition, in the study of actinomycetes, cannot be overemphasized. Numerous examples can be cited about conspicuous differences obtained with a slight change in the composition of the medium. The production of pigment, which was used to a great extent by previous investigators in classifying these organisms, is almost entirely dependent upon the composition of the medium (as well as upon other factors to a smaller extent); this was one of the chief reasons why different investigators described the same organism under different names. If the proper substances are offered in the proper forms, differences in concentration of these substances do not affect the growth of the cultures to such an extent.

Nocard (31) was the first one to cultivate in 1888 an *Actinomyces* in pure culture. This organism was exclusively aerobic, did not modify the reaction of neutral bouillon, even if sugar was added; when kept for 4 months at 40°C. it could still grow vigorously on fresh media; 10 minutes at 70°C. was sufficient to destroy the virulence and vitality of the organism. Bostroem (4) made an exhaustive study on the cultivation of pathogenic actinomycetes. Mention should also be made of the work of Gasperini (17), Rossi-Doria (34) and Musgrave, Clegg and Polk (28). Rossi-Doria (34) and Sanfelice (35) studied the pathogenicity of the actinomycetes isolated from different sources and stated that some of them proved to be pathogenic. Wright (47), having isolated a number of pathogenic forms from men and animals, concluded from the similarity in morphology and difficulty of cultivation, that they were all one species (*A. bovis*), making a rather poor growth on milk, potato and coagulated egg, refusing to grow at room temperature and essentially anaerobic.

The cultural studies of actinomycetes by these and other investigators is of limited value due to the fact that only complex organic media were used, not

standard in composition. The more recent workers on this group of organisms, namely Krainsky (22), Waksman and Curtis (44) and Conn (10), have introduced synthetic inorganic media and have developed several of these, so that they would allow a characteristic growth which helps to differentiate these organisms. On media, which do not contain favorable nutrients, such as nitrates and ammonium salts as sources of nitrogen and saccharose as a source of carbon, the organisms grow rather slowly and have a tendency to spread, while on media containing more favorable nitrogen (asparagin, peptone, etc.) and carbon sources (dextrose, starch, glycerin, etc.), they have a tendency to pile up and often the growth in the last cases may not be so characteristic. The poor media seem to call forth a typical development of the organisms; these should not be taken to hold true for all of them. Gilbert (19) stated that admission of air, dryness and the presence of carbohydrates in the medium, favor spore formation.

Culture media

The following media have been used for the study of cultural and biochemical characters of the actinomycetes. A number of others, not given here, were tried, but the data were not reported when found uncharacteristic.

1. *Synthetic solution* (Czapek's, modified). K_2HPO_4 , 1 gm.; $MgSO_4$, 0.5 gm.; KCl, 0.5 gm.; $FeSO_4$, 0.01 gm.; $NaNO_3$, 2 gm.; saccharose, 30 gm.; (in some instances saccharose was replaced by another carbohydrate, 30 gm. per liter); distilled water, 1000 cc. (a better growth is obtained by most species, when glycerin (30 gm.) is substituted for saccharose).

2. *Synthetic agar* (based on modified Czapek's solution). Same as above, with the addition of 15 gm. of agar per liter. This medium, together with the two media of Krainsky, was found to give the best results for the study of the morphology and cultural characters of the actinomycetes. Only those organisms that produce invertase make an abundant growth on this medium, but since the invertase-producing species were found to be few in number, the growth is rather scant, particularly on repeated transfer, and for that very reason characteristic, since a good development of the aerial mycelium takes place. The cultures should not be grown on this medium continuously, since they will tend to die out.

3. *Dextrose nitrate agar and glycerin nitrate agar*. In these either dextrose (30 gm.) or glycerin (30 gm.) is substituted for saccharose in the above medium. These two media will allow a much heavier growth of the organisms to take place, and will also give a characteristic growth of some species, but not all of them.

4. *Dextrose agar* (Krainsky's, p. 688). Dextrose, 10 gm.; K_2HPO_4 , 0.5 gm.; asparagin 0.5 gm.; agar, 15 gm.; distilled water, 1000 cc.

5. *Calcium malate agar* (Krainsky's, p. 679) with the addition of glycerin as suggested by Conn (10) (*malate-glycerin agar*). Calcium malate, 10 gm.; NH_4Cl , 0.5 gm.; K_2HPO_4 , 0.5 gm.; glycerin, 10 gm.; agar, 15 gm.; distilled water, 1000 cc.; reaction adjusted by use of NaOH to P_H 7.0.

6. *Egg-albumin agar*. Dextrose, 10 gm.; K_2HPO_4 , 0.5 gm.; $MgSO_4$, 0.2 gm.; $Fe_2(SO_4)_3$, trace; egg albumin, 0.15 gm.; agar, 15 gm.; distilled water, 1000 cc. The egg albumin is dissolved in N/10 NaOH until neutral to phenolphthalein, then added to the warm medium.

7. *Glycerin asparaginate agar* (Conn (10), p. 13). Dextrose, 1 gm.; glycerin, 10 gm.; sodium asparaginate, 1 gm.; $NH_4H_2PO_4$, 1.5 gm.; $CaCl_2$, 0.1 gm.; $MgSO_4$, 0.2 gm.; KCl, 0.1 gm.; $FeCl_3$, trace; agar, 12 gm.; distilled water, 1000 cc. Reaction adjusted by the addition of 8 cc. N/10 NaOH. The last two media were found to be very good for the isolation of the organisms, but not for cultural studies.

8. *Nutrient agar*. Peptone, 10 gm.; Liebig's extract, 5 gm.; NaCl, 5 gm.; agar, 20 gm.; distilled water, 1000 cc. Reaction adjusted to P_H 7.0 to 7.2. The addition of 1-2 per cent of glycerin to this agar makes it excellent for the growth of these organisms (can be used for carrying on cultures).

9. *Glucose broth*. Glucose, 10 gm.; peptone, 10 gm.; Liebig's meat extract, 5 gm.; NaCl, 5 gm.; distilled water, 1000 cc. Adjusted to P_H 7.0 to 7.2.

10. *Egg media*. Whole egg (unless otherwise stated) mixed, by means of a sterile spatula in a sterile container, tubed into sterile test tubes, coagulated and sterilized at 80 to 85°C. for 1 hour on 3 consecutive days. The addition of glycerin, as in the case of the Lubenau's or Petroff's medium, makes the growth, in some cases, more characteristic. The introduction of gentian violet in Petroff's (32) medium does not interfere with the growth, thus suggesting its use for isolation of pathogenic forms.

11. *Loeffler's blood serum*, prepared according to the standard formula (three-fourths beef serum and one-fourth glucose bouillon), coagulated and sterilized as egg media.

12. *Blood agar*. Ten per cent of rabbit blood added to sterile, redissolved and cooled nutrient agar; tubed, slanted or plated, and incubated for 48 hours to insure sterility.

13. *Potato plugs*, prepared in the usual manner and placed in test tubes, having a piece of glass rod on the bottom.

14. *Carrot plugs*, same as potato plugs.

15. *Starch agar* (27). Ten grams of starch were suspended in 800 cc. of water and boiled until the volume was reduced to 500 cc.; 500 cc. of the medium having the following composition: K_2HPO_4 , 1 gm.; $MgSO_4$, 1 gm.; NaCl, 1 gm.; $(NH_4)_2SO_4$, 2 gm.; $CaCO_3$, 3 gm.; agar, 10 gm.; tap water, 500 cc. were added and the medium completed as usual.

16. *Cellulose agar*. To 500 cc. of cellulose solution prepared by the method of McBeth and Scales (27) was added 500 cc. of a medium having the same composition as in 15, omitting the carbohydrate.

17. *Skimmed milk*. Fresh milk, separated and sterilized at 10 pounds for 30 minutes or on 3 consecutive days in flowing steam. Brom cresol purple was used for the study of the changes in reaction, according to Clark and Lubs (6).

18. *Gelatin*. Fifteen per cent of gold-label gelatin in distilled water. Reac-

tion usually unadjusted (about P_H 6.2); when 1 per cent starch was added in some cases to the gelatin, the medium was termed starch gelatin.

19. *Tyrosinate agar* for the study of the presence of tyrosinase. Glucose, 10 gm.; tyrosin, 1 gm.; $(NH_4)_2SO_4$, 0.5 gm.; K_2HPO_4 , 0.5 gm.; agar, 15 gm.; distilled water, 1000 cc., made neutral with NaOH.

The synthetic solution No. 1 was used as a basis for the study of the availability of carbon in different organic forms; by substituting either glycerin or dextrose in place of saccharose, the same medium was used for the study of availability of nitrogen in different compounds.

Effect of temperature

Gilbert (19) in 1904 isolated an Actinomyces from the soil which had an optimum temperature of 55°. Domec (12) found that the mycelium of *A. bovis* was destroyed when kept for 5 minutes at 60°, while the spores were destroyed at only 75° for 5 minutes. Foulerton and Jones (16) stated that 75° is the thermal death-point for all actinomycetes spores (*A. luteola* surviving at 75° for 20 minutes and killed in 30 minutes, the mycelium surviving at 60° for 20 minutes and killed at 70° for 20 minutes), 45° is the death-point for some actinomycetes. Acosta and Grande Rossi (1) isolated an Actinomyces (*A. invulnerabilis*) that withstood 6 discontinued sterilizations at 100° and was able to withstand temperatures of 130° to 160°. Lutman and Cunningham (26) found little or no difference between the thermal death-point of the mycelium and conidia of *A. scabies*; this point was found to be between 50° and 54°, while the optimum temperature was 25°. Krainsky (22) found that only *A. citreus* had its optimum at 26°, most actinomycetes grew best at 30° and some at 35°. The maximum for most species is 40°, for *A. diastaticus* alone between 45 and 50°. The minimum was below 18 to 20°. Most of the work reported in the following pages was carried out at 25°, unless otherwise stated. It is interesting to note that the animal pathogens grew much better at the higher temperatures (37°) than at the lower (25°).

Oxygen requirement

There seems to be some misunderstanding concerning the oxygen tension of the actinomycetes. Some of the older investigators stated that *Actinomyces bovis* is a strict aerobe, others claimed it to be strictly anaerobic (Wright), while still others found it half anaerobic. Musgrave and associates (28) stated that the fact of occurrence of strict anaerobes or aerobes is based upon errors of technique, since in no instance have they obtained a strict anaerobe or aerobe. Beijerinck (2) classified the actinomycetes as facultative anaerobes. The writer could obtain no growth of the organisms isolated from the soil when grown under strictly anaerobic conditions. At the same time some species are found, as will be shown later, that are able to grow deeply into the

medium, while others limit their growth to the surface. This would seem to indicate that the actinomycetes are not strict anaerobes, but some may be able to thrive under semi-anaerobic conditions.

Character of growth

Nadson (29) already pointed out that the term colony is used incorrectly in designating a mass of growth of an *Actinomyces*, since it is not true to nature to call a mass of mycelium developing out of a spore a colony, as in the sense of a bacterial colony. In the case of bacteria, we have colonies of groups of organisms, while in the case of actinomycetes, we have only one organism developing an extensive mycelium, therefore the term "pseudocolony" should rather be used. In the following pages the so-called colony, the growth below and above the substratum, will be termed "growth," "mass of growth" or "colony," as the term is commonly applied. In designating the amount of growth as well as other activities of the organisms, 1 means faint or scant, 2 fair, 3 good, 4 very good, and 5 excellent.

The single-celled *Actinomyces* colony is usually round and develops in the form of a semi-circle into the medium; most of the species form elastic-like colonies, which cannot be easily broken and are lifted by the needle out of the agar; the surface is usually dry and often presents a conical appearance, particularly when covered with the aerial mycelium; growing hyphae developing into the medium present, on detailed study (with magnifying glass), the typical character of the development of a mold, particularly in young cultures. The surface is usually covered with an aerial mycelium, either cottony, powdery, or smooth, either covering the entire surface or only in patches. The type of mycelium as well as spore formation is shown in the plates. Several species, such as *A. verne* and *A. bobili* never produce any true aerial mycelium, but form a heavy folded or lichnoid growth, which changes very little with age; other forms, such as *A. lavendulae*, *A. fradii*, *A. albosporus* may lose their ability to form the aerial mycelium when grown continuously on Medium No. 2, which is rather poor for making a good growth; when transferred upon other media they regain this property; while still others, notably *A. halstedii*, may lose entirely, upon continued cultivation on artificial culture media, their power to produce any aerial mycelium at all, and all attempts to bring it back to the original condition have failed so far. It should be noted here that all the cultures, unless otherwise stated, were incubated at 25°C. for 15 days.

Physiological action upon the media

Certain physiological effects of the growth of the actinomycetes upon media that were found to be significant and would help in separating the different organisms, are given. But one should always keep in mind the great variability of these organisms; certain characters may be changed on continued cul-

tivation on artificial culture media; some of these may even be characteristic of the species, such as the production of aerial mycelium, color of the potato plug, or pigmentation of gelatin. These characters can, in many cases, be regained, when the organism is grown on natural substrata and on favorable culture media; if not, one character should not be of sufficient importance to separate the strain into a different group; attention will be called to these facts later. This is the reason why it is so hard to identify an Actinomyces from a description, when not sufficient information is given.

The data observed in repeated series of culture are as follows: changes produced on milk, gelatin, egg and serum media, including liquefaction of solid media, changes in reaction (hydrogen-ion concentration) and pigment production. Studies were also made of the utilization of carbon and nitrogen compounds, proteolytic and diastatic action, growth on cellulose, reduction of nitrates and production of enzymes for all or few species. Odor production was made use of in the first paper, but it does not seem to be characteristic merely of a species, but of the whole group, and it is probably more of a qualitative rather than quantitative nature, depending on many factors.

Milk. The action upon milk seems to be quite characteristic of the species, although too many variations will be found here. The organism may coagulate the milk with a different speed. An organism may hydrolyse (clear) the milk, without any previous coagulation; coagulation may take place here too, but the coagulum is either too soft to be detected easily, or the clotting takes place in the form of fine flakes falling to the bottom which may easily be overlooked, as was found in several instances. Other organisms may not produce any visible changes in the milk. The proteolytic action was followed by the determination of amino-nitrogen of the substratum, using the micro-apparatus of Van Slyke. The change of reaction was studied by means of brom cresol purple, which is much superior for this work to litmus or azolitmin; this was prepared and added to the milk according to the method outlined by Clark and Lubs (5). The milk cultures were all grown at 37°. In designating the proteolytic action on milk, the following terms were used: very faint, when less than 10 per cent of the protein and other nitrogen of the milk has been transformed into amino-nitrogen in 40 days; faint or scant, 10-20 per cent; fair, 20-30 per cent; good, 30-40 per cent; very good, 40-50 per cent; excellent, above 50 per cent. In designating the final reaction of the milk, 0 means unchanged, 1 faintly alkaline, 2 fairly alkaline, 3 distinctly alkaline and 4 and 5 strongly or most alkaline with the particular indicator. Peptonization refers to the digestion of the clot previously formed, while hydrolysis refers to the clearing of the milk, without any visible clot formation. The amino and ammonia nitrogen were determined, in the case of coagulation of the milk, only on the liquefied portion, unless otherwise specified. Some organisms digest the precipitated casein so thoroughly that on the addition of acetic acid no precipitate is obtained, as in the case with *A. griseus*, *A. 206*, *A. poolensis*, *A. flavovirens*, *A. chromogenus* 205, and *A. griseolus*. The growth on the milk

was determined only at 25°, since very little of the growth itself was produced at 37°.

Gelatin. The liquefaction of gelatin cannot be used as a distinguishing character of the actinomycetes, because they all, with very few exceptions (*A. asteroides*), liquefy gelatin. The rapidity of liquefaction and the production of a soluble pigment are characteristic. Even here we find certain limitations: the temperature of incubation, length of cultivation of the organism and media on which it was grown previously, affect to some extent the rapidity of liquefaction, but, allowing for this, we find this characteristic of most species. The production of a brown pigment in the liquefied portion which spreads often into the unliquefied portion of the gelatin is characteristic of the species, with very few exceptions, when this ability is lost on continued cultivation upon artificial culture media. Few organisms produce a faint yellow to golden pigment; a green and a blue pigment were once obtained, but those organisms were lost during the course of the work.

In nearly all cases a 15 per cent solution of gelatin in distilled water was used, the reaction usually left unchanged. In one series of comparative culture, 1 per cent of starch was added to the gelatin, to study the effect of available carbohydrates upon the proteolytic action upon gelatin. The temperature of incubation is very important, since changes in temperature (between 18° and 25°C.) may affect the rapidity of liquefaction by the different organisms, affecting the rate of growth and thus affecting the comparative value of the data obtained. The cultures were grown either in Petri dishes or in tubes of equal diameter, and either the width of the liquefied zone in the dish or the height of the liquefied portion in the tube was measured at the end of a definite period (15–30 days at 18°). The liquefaction is often designated as rapid, medium and slow, depending on the width of the zone or height of the liquefied portion in the tube. The gelatin is either rapidly peptonized and a freely mobile fluid results (*A. albosporeus*, *A. griseus*, *A. flavorirens*, etc.), or gradually softened, the medium becoming thickly viscid (*Actinomyces* 205, *A. aureus*), which is true of the rather weakly proteolytic organisms.

Hydrolysis of starch. Starch was found to be, as will be shown later, one of the best sources of energy for the actinomycetes. The hydrolysis of starch was studied by three different methods. The saccharose in the synthetic solution was replaced by 1 per cent starch, the medium distributed in 50-cc. portions in flasks, sterilized and inoculated; at the end of 7, 14, 21 and 28 days, the solution was tested with iodine solution for the presence of starch. The second method was the starch plate, the formula for the medium having been given above; at the end of a definite period of time, a solution of iodine in potassium iodide was poured over the plate, and the width of the clear zone measured. The third method consisted in placing the synthetic solution containing 1 per cent starch, in place of saccharose, in test tubes of equal diameter, 10 cc. to a tube, the line between the hydrolized and unhydrolized starch being readily recognized. At the end of a definite period of incubation, the height

of the starch in the inoculated tube was compared with that of the uninoculated tube serving as control, this height measured serving as an indication of the rapidity of hydrolysis of starch or diastatic action. A portion of the clear liquid was withdrawn with a pipette and analyzed for starch and sugar. Most actinomycetes possess a very strong diastatic power both amylolytic and saccharogenic, hydrolyzing the starch to sugar; in some cases the hydrolysis is incomplete, since only the erythroreaction was obtained. Foulerton and Jones (16) reported that the 10 pathogenic species studied did not exhibit any diastatic action upon starch. This could be confirmed only in the case of *A. asteroides*, while the *A. hominis*, *A. madurae* and *A. bovis* exhibited marked diastatic action upon starch much similar to the saprophytic species. This difference may be due perhaps to the continued cultivation on artificial culture media. In describing the diastatic action of an organism, faint (1) designates a clear zone obtained on the starch plate in 12-15 days at 25°, 3-4 mm. wide; fair (2) 5-8 mm. wide; good (3) 10-15 mm.; very good (4) 15 mm. and more.

Action on cellulose. A number of methods were used for the study of the action of actinomycetes upon cellulose, but none of them was found satisfactory, some organisms giving a better reaction on one medium, and others on another. The plate method allowed a good growth of most organisms, and clear zones were obtained with several species, namely *A. violaceus-ruber*, *A. bobili*, *A. exfoliatus* and *A. albus*. Krainsky (22) objected to the use of this method, stating that the clear zone may be due to the solubility of the CaCO_3 or the phosphate through the action of the actinomycetes. It is true that this medium is not so suitable for the study of actinomycetes as for the study of cellulose-splitting bacteria, because the former will grow on this medium, even without the cellulose, on the agar alone. The methods suggested by Krainsky are not much better, since most species refuse to grow on his media at all. The ability to grow on cellulose was demonstrated for several cultures, by merely inserting a piece of filter paper or adding some reprecipitated cellulose to the synthetic solution placed in tubes, then sterilizing and inoculating. The interesting part of it is that few cultures that seemed to have attacked cellulose by one method did not do so by the others; this may be explained by the variation in the composition of the media.

Pigment formation. A number of pigments are produced by actinomycetes. Some of them are insoluble in water, remain in the cells and color the colony or mycelium, while others are soluble and diffuse into the solution or agar, the latter media being more favorable for the pigment production. The complex organic media, such as glucose broth and nutrient agar, are not favorable for the pigment formation. The production of a brown pigment chiefly on organic media which was thought to be characteristic of the *chromogenus* species is the property of several species. It was pointed out in a previous investigation by the author and Curtis (44), as well as by Krainsky (22), that there are many forms which are characterized by the production of a brown to dark

brown pigment on protein media; these species usually show also the chinon reaction on gelatin. Krainsky found that *A. flavo-chromogenus* produced the strongest chinon reaction; this species was not isolated by the writer, but *A. chromogenus* 205 shows the strongest reaction in our series. Gelatin is at first liquefied, then a chinon-gelatin compound is formed which is insoluble. This was not observed with the other chromogenus species. *A. aureus* and *A. laven-dulae* would also belong to the chromogenus types, since they produce the same characteristic brown pigment on protein media and attack proteins rather slowly. *A. bobili*, *A. ruber*, *A. flavus* and a few others also produce brown pigments on gelatin and other protein media, but they liquefy the gelatin rapidly.

All the so-called chromogenus species color potato black; this pigment production is ascribed to the enzyme tyrosinase. On incubating all the organisms on the tyrosin agar plate, out of 10-15 species producing brown pigments on proteins and potato plug, only some strains of *A. scabies* formed a dark brown spreading pigment and *A. chromogenus* 205 formed a lighter brown pigment, thus showing that the production of a brown pigment alone on gelatin, potato or other media is no sufficient proof of the identity of the organism.

Beijerinck (3) has shown that by symbiotic action of an Actinomyces with a common soil bacterium, tyrosin in an agar plate culture is oxidized to melanin which appears as black spots on the culture plate. Neither organism alone oxidized tyrosin to the same stage. Other species of Actinomyces produce blue, red or yellow pigments, the simultaneous presence of certain varieties of hay bacteria being favorable in the case of blue and red. Dextrose, malates and nitrates form the chromogeneous food in this case instead of tyrosin. It is considered that the Actinomyces produces homogentisic acid from tyrosin, and that the bacterium oxidizes this acid to melanin. The fact should be kept in mind that gelatin does not contain the amino acid tyrosin, and, since most Actinomyces that produce brown pigments on other media, produce it also on gelatin, we must conclude that the brown pigment is not always due to the production of dark substances (melanins) from tyrosin by the action of a tyrosinase. There seem to be two types of brown pigments: one produced by several species on protein media including gelatin and some pure amino acids, plant media (potatoes, carrots), and synthetic media, and another produced on protein media by organisms which produce dark pigments from tyrosin, as in the case of *A. scabies* and *Actinomyces* 205. Of the soluble pigments only one was studied, namely the red and blue pigment of *A. violaceus-ruber*, which is very interesting. This pigment consists of two or more pigments, which have been definitely demonstrated. One of these pigments acts as an indicator, which is red in acid media and blue in alkaline, the change taking place at about P_H 7.6. On synthetic agar, the organism produces at first a red pigment, since that medium is acid to the indicator (P_H 7.0); with the growth of the organism, the medium changes to alkaline and the pigment turns blue.

Reaction. The methods of study of fermentation of carbohydrates used in the separation of certain bacteria are inapplicable to the actinomycetes: they do not produce any gas and the change of reaction depends more on the source of nitrogen than that of carbon. The reaction was determined in all cases by means of the colorimetric method, the sulfonephthalein series of indicators being used, as recommended by Clark and Lubs (5).

Reduction of nitrates. The reduction of nitrates is a property of nearly all the actinomycetes, differing in a qualitative rather than in a quantitative way, the amount of reduction depending on the source of carbohydrate present in the medium. NaNO_3 was used invariably as the source of nitrate and the amount of nitrite produced was determined by a mixture of sulfonilic acid dissolved in 33 per cent acetic acid and α -naphthylamin, as usually given in the text books. It is characteristic that the reduction of the nitrates took place only to nitrites and not to ammonia or atmospheric nitrogen, at least the latter two have never been demonstrated. Krainsky (22) has already called attention to the fact that, although an active reduction to nitrites was shown only for a few species, many others do it, but the amount of reduction is so small that the nitrite is used up by the organism as soon as formed. The author (43) has shown elsewhere that certain organisms, such as *A. violaceus-ruber*, reduce nitrates very actively with all sources of carbon, while others do not show any reduction with most carbon compounds and only small quantities with others. The members of the first group usually assimilate nitrites very readily, while the members of the other may not. But there is no sharp line of demarkation between these two groups, with many organisms coming intermediate. It should be noted here that not the actual formation but the accumulation of nitrites was necessarily measured.

Proteolytic action. The proteolytic action of the actinomycete was measured in most instances by the amount of amino nitrogen or ammonia accumulated as a result of the action of the organism upon a given protein. The amino nitrogen was measured by the micro-apparatus of Van Slyke (38) and the ammonia by the Folin aeration method (15); in this way we can follow the splitting of the protein. Of course, we do not get the total amino nitrogen produced, since some of it is used up by the organisms and some transformed into other nitrogen compounds, such as ammonia. In determining the amino nitrogen present, when an amino acid is offered as a source of nitrogen, we can follow the process of utilization of the given source of nitrogen. The value of the determination of ammonia as an index of proteolytic action has been greatly overestimated by many bacteriologists, since it is probably a waste product in protein metabolism and depends on different factors. This has been discussed by the writer elsewhere (42). In differentiating the utilization of a definite compound from proteolytic action, the following points should be kept in mind: the amount of growth produced was taken as a measure of utilization, while the splitting of the protein, as measured by the amino and ammonia nitrogen (residual) was taken as a measure of proteolytic action.

Enzyme production. The following enzymes were studied: rennet-like enzymes, protease, invertase, diastase, cellulase (or cytase) and tyrosinase.

Some of the data on the metabolism of this group of organisms that may have a direct bearing upon the separation of the different members of the group will be given at the end of this paper, under "Cultural and biochemical studies."

DESCRIPTION OF THE SPECIES

Actinomyces alboflavus Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: None; mycelium is straight, branching.

Calcium malate agar: Straight, branching mycelium, with very little tendency to form spirals.

2. Conidia.

Synthetic agar: Very few oval-shaped conidia observed.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Glossy, spreading, colorless at first, later becoming yellowish.

Aerial mycelium: White powdery, with yellow tinge; property nearly all lost with the growth of culture on artificial media.

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Glossy, restricted, limited to surface, light pinkish-cinnamon color (Rdg. XXIX, 15"-d).

Aerial mycelium: None in 15 days; faint powdery white sprinkling in 30 days.

Soluble pigment: None.

3. Glucose agar.

Growth: Restricted surface growth, much folded; color creamy, with sulfur-yellow (Rdg. V, 25-f) tinge of surface.

Aerial mycelium: None.

Soluble pigment: None.

4. Nutrient agar.

Growth: Restricted, cream-colored.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood serum, 37°. No growth.

6. Egg-media, 37°. No growth.

7. Starch agar, 25°, 16 days.

Growth: Thin, yellowish colored, spreading surface growth.

Aerial mycelium: None.

Enzymatic zone: 12-14 mm. wide, hydrolysis incomplete.

8. Potato plug.

Growth: Moist, wrinkled, cream-colored growth is produced in 4 days.

Aerial mycelium: None, sometimes white mycelium is produced.

Color of plug: White.

9. Carrot, 25°, 22 days.

Growth: Numerous cream-colored colonies.

Aerial mycelium: Thin, powdery, white, all over surface, developing in 15 days.

Color of plug: Unchanged.

10. Gelatin, 18°.

Growth: Abundant, colorless, large, flaky colonies on bottom of liquefied portion.

Aerial mycelium: None or thin white.

Soluble pigment: None.

Liquefaction: Medium (1½–2 cm. in 35 days).

11. Synthetic solution.

Growth: Few small colorless colonies or flakes on glass and bottom of tube.

Aerial mycelium: None.

Soluble pigment: None.

12. Milk, 37°.

Growth (25°): Pinkish ring on surface.

Coagulation: None.

Hydrolysis: 10–12 days at 37°, while at 25° it is not completed in 30 days.

Change of reaction: Distinctly alkaline (3).

13. Glucose broth, 25°, 12 days.

Growth: White, cylindrical colonies, grown together on surface of liquid; on continued cultivation there is formed only a small flaky mass on bottom of tube.

Aerial mycelium: White.

Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrate formation:* Fair with different sources of carbon.

2. *Proteolytic action:* Very good on milk and glucose broth, fair on gelatin.

3. *Change of reaction:* Distinctly alkaline in milk; faint alkalinity in acid gelatin, in presence of starch, faint acidity in alkaline glucose broth.

4. *Inversion of sugar:* Positive; often negative results are obtained.

5. *Diastatic action:* Good; 1 per cent used up in 14 days; also good on plate, with incomplete hydrolysis (amylolytic action good, while saccharogenic action poor).

6. *Growth on cellulose:* Very scant.

Hab. New Jersey meadows and orchard and California upland soils.

Actinomyces albosporeus Krainsky, 1914, p. 687, emend Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: None; aerial mycelium forms straight branching hyphae; on further study some close spirals are found.

Calcium malate agar: Very little tendency to form spirals; branches straight.

2. Conidia.

Synthetic agar: Spherical and oval shaped, 1.0 to 1.8 x 0.8 to 1.2 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar; 15 days.

Growth: Spreading both on surface but chiefly into the medium; colorless at first with pink center, later (24 days) becoming brownish vinaceous (Rdg. XXXIV, 5'''-b).

Aerial mycelium: White patches at first, later covering all surface; often culture remains without aerial mycelium.

Soluble pigment: None.

2. Calcium malate-glycerin agar.
Growth: Thin spreading growth, with smooth entire edge; rose colored, with wide colorless margin.
Aerial mycelium: White, powdery, only in center of growth.
Soluble pigment: None.
3. Glucose agar.
Growth: Spreading surface growth, wrinkled and radiating toward periphery edge entire; color Acajou red (Red. XIII, 1'-i) with white colorless margin.
Aerial mycelium: None in 15 days; white patches appear in 30 days.
Soluble pigment: None.
4. Nutrient agar.
Growth: Minute, cream-colored colonies.
Aerial mycelium: None.
Soluble pigment: None.
5. Blood serum, 37°.
Growth: Restricted, pink-colored.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: None.
6. Egg-media, 37°.
Growth: Thin, spreading, wrinkled, gray growth in 6 days; with age of culture (30 days), a brownish tinge develops.
Aerial mycelium: None.
Soluble pigment: None.
7. Starch plate, 25°, 12 days.
Growth: Thin, spreading, transparent, with red tinge.
Aerial mycelium: None.
Enzymatic zone: Broad (20 mm. and more).
8. Potato plug.
Growth: Thin, spreading, slightly wrinkled in center; color of growth gray at first, later becoming brown and gray, with greenish tinge (30 days).
Aerial mycelium: None, sometimes white mycelium is produced.
Color of plug: Unchanged.
9. Carrot, 25°, 22 days.
Growth: Scant, restricted, folded, cream-colored, edges turning pink.
Aerial mycelium: None.
Color of plug: Unchanged.
10. Gelatin, 18°, 15 days.
Growth: Yellow, later changing to red color, with hyaline margin; mass of colorless flakes dropping to bottom of liquefied portion.
Aerial mycelium: None, sometimes **gray** patch.
Soluble pigment: None.
Liquefaction: Rapid (2-3 cm. of depth of tube liquefied in 35 days).
11. Synthetic solution.
Growth: Small pinkish flaky colonies.
Aerial mycelium: Rose-colored.
Soluble pigment: None.
12. Milk. No visible action on milk at both 25° and 37°.
Growth (25°): Pinkish scant surface ring.
Coagulation: None.
Hydrolysis: None.
Change of reaction: Unchanged.

13. Glucose broth, 25°, 12 days.

Growth: Pinkish ring on surface in contact with glass.*Aerial mycelium*: White, scant.*Soluble pigment*: None.

14. Utilization of different carbon compounds.

<i>Arabinose</i>	0	<i>Dextrose</i>	3	<i>Lactose</i>	2
<i>Glycerin</i>	1	<i>Saccharose</i>	1-2	<i>Maltose</i>	1
<i>Cellulose</i>	0	<i>Mannite</i>	4	<i>Starch</i>	4
<i>Organic acids</i>	1				

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Fair, with different sources of carbon.2. *Proteolytic action*: Faint on milk; good on gelatin, but only fair, when 1 per cent of starch is added; good on glucose broth.3. *Change in reaction*: Usually becomes slightly alkaline with NaNO_3 as source of nitrogen with different carbon compounds; distinctly alkaline in acid gelatin, but only faintly alkaline when 1 per cent of starch is present; faint acidity in alkaline glucose broth and no change of reaction in milk.4. *Inversion of sugar*: Positive, often negative.5. *Diastatic action*: Fair; 1 per cent starch not used up in 14 days; very good on starch plate, zone 20 mm. and more wide.6. *Growth on cellulose*: None to very scant.*Hab.* Upland California soil.*Actinomyces albus* Krainsky, 1914, p. 683, emend. Waksman and Curtis

This organism resembles in certain respects the one described by Krainsky and before by others, although it is doubtful, whether it is the same organism as the one that Krainsky described.

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: None; straight branched mycelium. A few short, closed spirals are found on the glycerin-synthetic agar.

2. Conidia.

Synthetic agar: Spherical and oval, 1.2 to 1.6 x 1.1 to 1.4 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar:

Growth: Abundant, spreading, grayish color.*Aerial mycelium*: White, covering all growth, produced in 4-5 days.*Soluble pigment*: None.

2. Calcium malate-glycerin agar.

Growth: Thin, penetrating deep into the medium, edge myceloid, gray colored.*Aerial mycelium*: Abundant, all over surface, pale mouse gray (Rdg. LI, 15^{mm}-d) with a large white edge.*Soluble pigment*: None.

3. Glucose agar.
Growth: Thick, surface growth slightly elevated in center, penetrating to some extent into medium; edge, myceloid, radial lines from center to periphery; color gray with yellowish center.
Aerial mycelium: Powdery, all over growth, except narrow edge, pale mouse gray color (Rdg. LI, 15^{mm}-d).
Soluble pigment: None.
4. Nutrient agar.
Growth: Glossy, cream-colored, spreading.
Aerial mycelium: Few white patches.
Soluble pigment: None.
5. Blood agar, 37°, 15 days.
Growth: Green colored, restricted, wrinkled.
Aerial mycelium: White, often none at all.
Soluble pigment: None.
Hemolysis: None.
6. Blood serum, 37°.
Growth: Thin, cream-colored smear appears early (4 days) and remains unchanged.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: None.
7. Egg-media, 37°.
Growth: Thin, spreading, wrinkled, of a cream color.
Aerial mycelium: None.
Soluble pigment: None.
8. Starch plate, 15 days.
Growth: Thin, spreading, transparent.
Aerial mycelium: None.
Soluble pigment: None.
Enzymatic zone: Broad (10-15 mm.).
9. Potato plug.
Growth: Abundant, much wrinkled, cream-colored, with a faint greenish tinge developing in 15 days.
Aerial mycelium: Thin, white, only covering edge of growth.
Color of plug: Purplish with age.
10. Carrot, 25°, 22 days.
Growth: Abundant, spreading, much folded, cream-colored.
Aerial mycelium: Very thin, white, powdery.
Color of plug: Unchanged.
11. Gelatin, 18°C.
Growth: Small, cream-colored masses on surface and throughout the liquefied portion.
Aerial mycelium: White patches.
Soluble pigment: Brown at first, but on continued cultivation on artificial culture media, power of pigment production is lost.
Liquefaction: Medium (1 cm. of depth of tube in 35 days).
12. Synthetic solution.
Growth: Small, white colonies on glass of tube, which may also form a surface pellicle.
Aerial mycelium: None or thin white.
Soluble pigment: None.

13. Milk, 37°.

Growth (25°): Brownish surface ring.

Coagulation: None.

Hydrolysis: Complete in 20 days, leaving clear solution.

Change of reaction: Strongly alkaline (5).

14. Glucose broth, 25°, 12 days.

Growth: White ring on surface, in contact with glass; also abundant, colorless flaky mass on bottom of tube.

Aerial mycelium: None to scant white.

Soluble pigment: None.

15. Utilization of different carbon compounds.

Arabinose.....	2	Dextrose.....	3	Lactose.....	3
Glycerin.....	3	Saccharose.....	2-3	Maltose.....	3
Cellulose.....	0	Mannite.....	3	Starch.....	3
Organic acids.....	1-2 (lactate)				

16. Utilization of different nitrogen compounds.

Ammonium sulfate.....	0-1	Ammonium carbonate.....	0
Sodium nitrite.....	1	Acetamide.....	1
Sodium nitrate.....	1-5	Leucin.....	3
Glycocoll.....	3-4	Casein.....	2-3
Asparagin.....	2	Fibrin.....	3
Egg-albumin.....	3-4	Urea.....	1
Peptone.....	2-4		

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Good, with starch as a source of carbon; none or very little, with saccharose and glycerin.
2. *Proteolytic action*: Good in milk; faint on gelatin, with practically none in the presence of starch.
3. *Change of reaction*: With NaNO_3 as a source of nitrogen and different carbon compounds, there may be no change, slight acidity or slight alkalinity; strongly alkaline in milk; distinctly alkaline in acid gelatin, in absence of available carbohydrates, only faintly alkaline in presence of starch; acid in alkaline glucose broth (pH 7.9 changed to 7.1).
4. *Inversion of sugar*: None.
5. *Diastatic action*: Good, 1 per cent starch being used up in 14 days; good on plate, zone 10-15 mm. wide in 15 days.
6. *Growth on cellulose*: No growth in solution with strips of paper as source of carbon; good growth on cellulose plate, with clear zone (1 mm.) around colony.

Hab. This organism was isolated from New Jersey orchard and garden, Colorado, Oregon and California adobe soils.

Actinomyces asteroides (Eppinger) Gasperini (Syn. *Streptothrix eppingeri* Rossi-Doria)

I. MORPHOLOGY.

1. Spirals.

No true spirals observed; the straight, fine mycelium may show the wavy effect or a few spirals may be produced.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.
Growth: Thin, spreading, penetrating deep into the medium, orange colored.
Aerial mycelium: None.
Soluble pigment: None.
2. Calcium malate-glycerin agar and glucose agar, 25°, 12 days.
Growth: Folded, irregular, light orange, chiefly on surface of medium.
Aerial mycelium: Faint white on calcium malate, none on glucose agar.
Soluble pigment: None.
3. Nutrient agar, 25°, 15 days.
Growth: Much folded, chiefly on surface, at first light yellow, later turning deeper yellow, yellowish red to almost orange colored.
Aerial mycelium: None or traces of white.
Soluble pigment: None.
4. Blood agar, 37°, 15 days.
Growth: Thin, brownish smear in 24-48 hours.
Aerial mycelium: Gray, appearing in 8-10 days.
Soluble pigment: None.
Hemolysis: None.
5. Blood serum, 37°.
Growth: None at first, later (15 days) thin, white smear is formed; often a good growth is formed in 2-3 days.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: None.
6. Egg-media, 37°.
Growth: Thin, faintly wrinkled, yellowish growth.
Aerial mycelium: Thin, white, all over growth.
Soluble pigment: None.
7. Starch plate, 25°, 15 days.
Growth: Restricted, scant, orange-colored.
Aerial mycelium: Thin, white.
Enzymatic zone: None.
8. Potato plug.
Growth: Much wrinkled, whitish at first, then yellow colored to almost brick red.
Aerial mycelium: None or fine powdery efflorescence.
Color of plug: Unchanged.
9. Carrot, 25°, 22 days.
Growth: At first (7 days), cream-colored, much folded, restricted growth, later turning orange-colored.
Aerial mycelium: None.
Color of plug: Unchanged.
10. Gelatin, 18°, 30 days; 37°, 10 days.
Growth: Yellowish.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: None.
11. Synthetic solution.
Growth: Pinkish flaky growth on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.

12. Milk, 37°, 20 days. Orange colored ring in contact with glass of tube, no visible transformation of the milk.

Coagulation: None.

Peptonization: None.

Hydrolysis: None.

Change of reaction: Unchanged.

13. Glucose broth, 25°, 12 days.

Growth: Thin, yellowish pellicle over entire surface of liquid.

Aerial mycelium: None.

Soluble pigment: None.

14. Utilization of different carbon compounds.

<i>Arabinose</i>	0	<i>Dextrose</i>	4	<i>Lactose</i>	2
<i>Glycerin</i>	2	<i>Saccharose</i>	2-3	<i>Maltose</i>	3
<i>Cellulose</i>	1	<i>Mannite</i>	2	<i>Starch</i>	1
<i>Organic acids</i>	1-3 (lactate)				

15. Utilization of different nitrogen compounds.

<i>Ammonium sulfate</i>	0	<i>Ammonium carbonate</i>	0
<i>Sodium nitrate</i>	0	<i>Acetamide</i>	1
<i>Sodium nitrite</i>	1	<i>Leucin</i>	2
<i>Glycocoll</i>	2	<i>Peptone</i>	2
<i>Asparagin</i>	1	<i>Casein</i>	1-2
<i>Egg-albumin</i>	3	<i>Fibrin</i>	1

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair to excellent, depending on source of carbon; the better the carbon source is for the growth of the organism, the larger are the amounts of nitrites accumulated.
2. *Proteolytic action:* Very faint on milk, gelatin and most other proteins.
3. *Change of reaction:* Unchanged, acid (mannite, glycerin), or alkaline (organic acids and a few others) with NaNO_3 as source of nitrogen; distinctly acid with the different amino acids and proteins used and glycerin as a source of carbon; faint alkalinity in glucose broth.
4. *Inversion of sugar:* Negative.
5. *Diastatic action:* None, both on plate and tube.
6. *Growth on cellulose:* None.

Hab. Received from Dr. K. F. Meyer, of the Hooper Institute, San Francisco, Cal., who received it from the Pasteur Institute in 1914.

Actinomyces aureus Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: Numerous long spirals, 17 to 20 x 4 to 5 μ .

Dextrose agar: Few spirals of open type, although most of the aerial mycelium shows the curving tendency; the spirals are sinistrorse, 3.5-4.5 μ in diameter.

2. Conidia.

Synthetic agar: Abundant, spherical to oval, 0.6-1.0 x 0.8-1.4 μ (1.0-1.2 x 1.0-1.5).

Dextrose agar: Elliptical to oval, 0.5-0.8 x 0.8-1.4 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 15 days.

Growth: Spreading, thin, colorless, developing deep into the medium.

Aerial mycelium: Thin, powdery, at first mouse-gray, later cinnamon drab (Rdg. XLVI, 13'''); ring formation in the aerial mycelium is prominent.

Soluble pigment: None; reverse of growth becomes dark brown.

2. Calcium malate-glycerin agar, 15 days.

Growth: Spreading, cream-colored, growth penetrating extensively into the medium; in 35 days, surface growth is almost black.

Aerial mycelium: Thin powdery, all over surface, leaving only narrow margin uncovered (portion below surface of medium), of an hair brown (Rdg. XLVI, 17'''-i) color, with a white edge.

Soluble pigment: None.

3. Glucose agar, 15 days.

Growth: Spreading surface growth, of a light orange color, also penetrating into medium; center elevated, margin hyaline.

Aerial mycelium: Powdery, covering nearly all colony, leaving bare edge, of a light drab color (Rdg. XLVI, 17'''-b).

Soluble pigment: None.

4. Nutrient agar, 15 days.

Growth: Restricted, gray-colored.

Aerial mycelium: None.

Soluble pigment: Deep brown, spreading.

5. Blood agar, 37°, 7 days.

Growth: Dark brown, glossy, restricted, folded.

Aerial mycelium: None.

Soluble pigment: Narrow, dark zone around growth.

Hemolysis: None.

6. Blood serum, 37°.

Growth: Restricted, cream-colored growth; often no growth at all.

Aerial mycelium: None.

Soluble pigment: Dark zone around growth.

Liquefaction: None.

7. Egg-media, 37°.

Growth: Thin, wrinkled, brownish-colored growth.

Aerial mycelium: None.

Soluble pigment: Narrow, purple zone around growth.

8. Starch plate, 25°, 12 days.

Growth: Thin, spreading, transparent.

Aerial mycelium: Buff-colored, all over surface, in zone formation.

Enzymatic zone: Fair, 8-10 mm.

9. Potato plug, 25°, 10 days.

Growth: Abundant, much wrinkled, brown-colored, later (15 days) becoming black with gray margin.

Aerial mycelium: Thin, white patches, later becoming ash-gray.

Color of plug: Black.

10. Carrot, 25°, 22 days.

Growth: Thin, restricted, gray, turning purplish brown.

Aerial mycelium: None.

Soluble pigment: Black.

11. Gelatin, 18°, 30 days.

Growth: Fair, cream-colored, changing to brown, spreading.*Aerial mycelium*: Usually none; white aerial mycelium may be produced at times, particularly in exposed portion of growth.*Soluble pigment*: Brown, spreading into unliquefied portion.*Liquefaction*: At first rapid, later slow.

12. Synthetic solution.

Growth: Flakes throughout the medium; light powdery colonies on surface.*Aerial mycelium*: Mouse-gray.*Soluble pigment*: None.

13. Milk, 37°. Soluble brown pigment. No visible action on milk at 37°.

Growth (25°): Black surface ring.*Coagulation*: None, only thickening of milk may often be observed.*Hydrolysis*: In certain cases some digestion is found; in most cases, no visible action upon the milk.*Change of reaction*: Unchanged to faintly alkaline in digested tubes.

14. Glucose broth, 12 days, 25°.

Growth: Thin brownish ring on surface in contact with glass; flaky mass on bottom of tube.*Aerial mycelium*: None.*Soluble pigment*: Deep brown.

15. Utilization of different carbon compounds.

Arabinose.....	0	Dextrose.....	4	Lactose.....	3-4
Glycerin.....	3	Saccharose.....	1-2	Starch.....	2
Cellulose.....	0	Organic acids.....	1		

16. Utilization of different nitrogen compounds (glycerin as source of energy).

Ammonium sulfate.....	1-5	Ammonium carbonate.....	1-2
Sodium nitrite.....	3	Acetamide.....	2
Sodium nitrate.....	3-5	Leucin.....	4-5
Glycocoll.....	5	Casein.....	4-5
Asparagin.....	4	Fibrin.....	3-4
Egg-albumin.....	4-5	Urea.....	1
Peptone.....	5		

When glycerin is replaced by dextrose, the ammonium salts and the amides are utilized to a much greater extent.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Only with starch and glycerin as sources of carbon, not with saccharose.
2. *Proteolytic action*: Proteolytically this organism is not very active; it exerts a fair action on milk, gelatin and glucose broth.
3. *Change of reaction*. Usually slightly alkaline with NaNO_3 as a source of nitrogen and different sources of carbon; distinctly alkaline in acid gelatin, both in presence and absence of starch (from pH 6.2 to pH 7.6 and 7.4); very distinctly acid in alkaline glucose broth (pH changed from 7.9 to 5.8 and less).
4. *Inversion of sugar*: None to positive.
5. *Diastatic action*: Good; 1 per cent starch nearly all used up in 14 days; fair on plate, zone 8-10 mm. wide.
6. *Growth on cellulose*: Very good by plate method (no zone formation); but none on paper or precipitated cellulose in solution.

Hab. This is one of the most common organisms, or rather groups of organisms found in the soil; a number of strains have been isolated, which vary in some details but run into one another; the whole group is related to the *chromogenus* species. Isolated from New Jersey garden, orchard, meadow and forest soils, Iowa, Louisiana, North Dakota, Hawaii, Texas, Alaska and Colorado soils.

Actinomyces bobili Waksman and Curtis

I. MORPHOLOGY.

1. Synthetic agar.

The branching of the hyphae is often so close as to have the appearance of whirls. No spirals.

2. Glycerin-synthetic agar.

Few close spirals of a dextrorose type.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Abundant, glossy, wrinkled, chiefly on surface, elevated, with lichenoid margin, at first coral red (Rdg. XIII, 5'), later becoming Acajou red (Rdg. XIII, 3').

Aerial mycelium: None at first; when culture is grown longer on artificial media, scant white mycelium develops.

Soluble pigment: None.

2. Calcium malate-glycerin agar, 12 days.

Growth: Spreading, both on surface and into medium; color cinnamon-buff (Rdg. XXIX, 17''-b).

Aerial mycelium: None.

Soluble pigment: None.

3. Glucose agar.

Growth: Restricted, finely wrinkled, chiefly on surface of medium; color coral red (Rdg. XIII, 5'), with hyaline margin.

Aerial mycelium: None.

Soluble pigment: None.

4. Nutrient agar.

Growth: Restricted, glossy, at first gray, later becoming brownish.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood serum.

Growth: Minute, glossy, gray colonies.

Aerial mycelium: None.

Soluble pigment: Brown zone around growth.

Liquefaction: None.

6. Egg-media.

Growth: Thin, wrinkled, at first (4 days) brown, later (15 days) turning jet black.

Aerial mycelium: None.

Soluble pigment: Narrow purple zone around growth.

7. Starch plate, 25°, 16 days.

Growth: Spreading, pink colored.

Aerial mycelium: White traces in isolated spots.

Enzymatic zone: 10-11 mm., hydrolysis incomplete.

8. Potato plug.
Growth: Thin, at first yellowish, later (15 days) turning red; surface dry and wrinkled.
Aerial mycelium: Red, with some scant white.
Color of plug: At first unchanged, later (15 days) a black zone is formed around the growth.
9. Carrot, 25°, 22 days.
Growth: Abundant, spreading, folded net-like, cream-colored.
Aerial mycelium: None.
Color of plug: Unchanged.
10. Gelatin, 18°, 30 days.
Growth: Dense cream-colored to brownish.
Aerial mycelium: None.
Soluble pigment: Brown.
Liquefaction: Rapid.
11. Synthetic solution.
Growth: Colorless, with orange center, often pinkish colonies through medium, collecting on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None to faint yellow.
12. Milk, 37°.
Growth: Dark brown surface ring; brown soluble pigment.
Coagulation: None.
Hydrolysis: Completed in 15-18 days both at 37° and 25°.
Change of reaction: Distinctly alkaline (3).
13. Glucose broth, 25°, 12 days.
Growth: Flakes on bottom and round colonies all throughout medium, chiefly in contact with glass.
Aerial mycelium: None.
Soluble pigment: None to brownish.
14. Utilization of different carbon compounds.
- | | | | | | |
|--------------------|---------------|-----------------|-----|--------------|---|
| Arabinose..... | 0 | Dextrose..... | 2 | Lactose..... | 3 |
| Glycerin..... | 3 | Saccharose..... | 1-2 | Maltose..... | 2 |
| Cellulose..... | 4 | Mannite..... | 0 | Starch..... | 3 |
| Organic acids..... | 1-2 (acetate) | | | | |

15. Utilization of different nitrogen compounds (glycerin as source of carbon).

Ammonium sulfate.....	0	Ammonium carbonate.....	0
Sodium nitrite.....	0	Acetamide.....	1-2
Sodium nitrate.....	1	Leucin.....	2-3
Glycocoll.....	2-3	Casein.....	3-4
Asparagin.....	1	Fibrin.....	3
Egg-albumin.....	1-3	Urea.....	1
Peptone.....	2-4		

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Very good, with many sources of carbon, although none or mere traces are obtained with saccharose and often with glycerin.
2. *Proteolytic action*: Very good in milk.
3. *Change of reaction*: Usually slight alkalinity, with NaNO_3 as a source of nitrogen.
4. *Inversion of sugar*: Positive.

5. *Diastatic action*: Very good; 1 per cent starch used up in 7 days; saccharogenic power on plate is not very good, since the hydrolysis of the starch is incomplete in 16 days.
6. *Growth on cellulose*: Good on plate and reprecipitated cellulose, but none on filter paper in solution; clear zone is formed on plate around colony.
- Hab.* Isolated from New Jersey garden and California adobe soils.

Actinomyces bovis Harz

I. MORPHOLOGY.

1. *Synthetic agar*: Thin, branching hyphae; few open spirals, of a dextrorose type.

II. CULTURAL CHARACTERISTICS.

1. *Synthetic agar*.

Growth: Restricted, developing deep into the medium, white, turning yellowish in 10-12 days.

Aerial mycelium: Appears late (20 days), light sulfur-yellow, powdery, covering all growth.

Soluble pigment: None.

2. *Calcium malate-glycerin agar*, 25°, 20 days.

Growth: Restricted, brownish growth chiefly in the medium.

Aerial mycelium: None.

Soluble pigment: None.

3. *Glucose agar*, 25°, 20 days.

Growth: Restricted yellowish, later becoming dark, consisting of a mass of small colonies, developing to some extent into the medium.

Aerial mycelium: Thin, sulfur-yellow.

Soluble pigment: None.

4. *Nutrient agar*.

Growth: Abundant, restricted on surface of medium, at first cream-colored, later becoming fawn-colored, brown, then almost black.

Aerial mycelium: Pale yellow green (Rdg. V, 27 f).

Soluble pigment: None.

5. *Blood agar*, 37°, 15 days.

Growth: Good, spreading.

Aerial mycelium: None.

Soluble pigment: None.

Hemolysis: Faint at first, 1-2 mm. zone in 15 days.

6. *Blood serum*, 37°, 15 days.

Growth: Slow, thin, gray growth, later turning yellowish.

Aerial mycelium: Sulfur-yellow.

Soluble pigment: None.

Liquefaction: Medium (12-15 days).

7. *Egg media*, 37°, 15 days.

Growth: Thin, cream-colored smear.

Aerial mycelium: Thin, white, chiefly over edge of growth.

Soluble pigment: None.

8. *Starch plate*, 25°, 15 days.

Growth: Growth is of a dirty yellowish color.

Aerial mycelium: None.

Enzymatic zone: Fair, 6-8 mm. wide.

9. Potato plug.
Growth: Abundant, much wrinkled, gray to canary yellow.
Aerial mycelium: Yellow (4 days), turning to characteristic sulfur-yellow (8 days).
Color of plug: Unchanged at first, later (17 days) turning brown.
10. Carrot, 25°, 22 days.
Growth: Fair, restricted, cream-colored, developing a dark reverse, wrinkled.
Aerial mycelium: Sulfur-yellow, powdery, all over surface, without leaving any margin.
Color of plug: Narrow dark zone.
11. Gelatin, 18°, 30 days.
Growth: Gray to brownish.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: Rapid at 37°, slow at 18°.
12. Synthetic solution.
Growth: None. When glycerin is substituted for saccharose, a few colorless flakes are formed on the bottom of the tube.
13. Milk, 37°.
Growth (25°): Thin yellowish surface growth.
Coagulation: 10-12 days.
Peptonization: Begins soon after coagulation is completed, proceeds somewhat slowly and is all completed in 40 days.
Hydrolysis: Often hydrolysis of the milk, without previous coagulation, may take place.
Change of reaction: Faintly (1) to distinctly alkaline (3).
14. Glucose broth, 25°, 12 days.
Growth: Small, round, flaky, colorless mass on bottom of tube; thin layer of a yellowish tinge on surface.
Aerial mycelium: Sulfur-yellow, scant.
Soluble pigment: None.
15. Utilization of different carbon compounds.
- | | | | | | |
|----------------------------|---|----------------------|---|------------------------|---|
| <i>Dextrose</i> | 2 | <i>Lactose</i> | 2 | <i>Glycerin</i> | 1 |
| <i>Saccharose</i> | 1 | <i>Maltose</i> | 1 | <i>Cellulose</i> | 0 |
| <i>Organic acids</i> | 1 | | | | |
16. Utilization of different nitrogen compounds.
- | | | | |
|-------------------------------|-----|---------------------------------|-----|
| <i>Ammonium sulfate</i> | 0 | <i>Ammonium carbonate</i> | 0 |
| <i>Sodium nitrite</i> | 0 | <i>Acetamide</i> | 1 |
| <i>Sodium nitrate</i> | 1 | <i>Leucin</i> | 2 |
| <i>Glycocoll</i> | 2 | <i>Casein</i> | 3-4 |
| <i>Asparagin</i> | 2-3 | <i>Peptone</i> | 2 |
| <i>Egg-albumin</i> | 3-4 | <i>Fibrin</i> | 2-3 |
| <i>Urea</i> | 1 | | |

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Excellent with carbon compounds that offer a good source of energy (saccharose, lactose, glycerin).
2. *Proteolytic action:* Good on milk; fair on peptone, casein, fibrin, egg-albumin and gelatin.

3. *Change of reaction:* Faintly alkaline in all cases, when NaNO_3 is the only source of nitrogen; distinctly alkaline with different proteins or amino acids as sources of nitrogen and glycerin as a source of carbon.
4. *Inversion of sugar:* Negative.
5. *Diastatic action:* Fair on plate.
6. *Growth on cellulose:* None or very scant, although good growth was obtained on sterile soil.

Hab. Received from Dr. K. F. Meyer, who had it from the American Museum of Natural History, received from Parke Davis Co., in 1911 (0.4); also received directly from Parke Davis Co., in 1918. The two cultures have shown somewhat different cultural characters; when received, the latter culture did not develop readily any aerial mycelium; but on continued cultivation upon synthetic media, both gave similar cultural and biochemical characters, which varied in their action in quantity rather than in quality.

Actinomyces californicus Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Narrow, long, open spirals, belonging to the corkscrew and sinistrorose type on the synthetic media.

2. Conidia.

Straight hyphae and spirals break up to form small spherical to oval spores.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 15 days.

Growth: Spreading, vinaceous colored (Rdg. XXVII, 1''-d), chiefly in the substratum; surface growth limited only to aerial mycelium.

Aerial mycelium: Powdery, thin light neutral gray (Rdg. LIII, N. G.-C), with distinct zonation.

Soluble pigment: None.

2. Calcium malate-glycerin agar, 15 days.

Growth: Restricted, cream-colored.

Aerial mycelium: Same as on No. 1.

Soluble pigment: None.

3. Glucose agar, 15 days.

Growth: Same as on No. 1, but not spreading so much into the medium.

Aerial mycelium: Same as on No. 1, but more abundant, no zonation, leaving uncovered margin.

Soluble pigment: None.

4. Nutrient agar.

Growth: Thin, restricted, yellowish to creamy in color.

Aerial mycelium: Powdery, covering all the surface of growth, white to cream-colored.

Soluble pigment: None.

5. Blood agar, 37°.

Growth: Gray, with tinge of red, restricted, folded.

Aerial mycelium: None.

Soluble pigment: None, often a dark reverse is obtained.

Hemolysis: None.

6. Blood serum, 37°.
Growth: Cream-colored, spreading.
Aerial mycelium: None at first, some white isolated spots appearing in 10-15 days.
Soluble pigment: None at first, with a faint brown pigment developing in 10-15 days.
Liquefaction: Faint, not increasing with age of culture.
7. Egg-media, 37°, 3 days.
Growth: Cream-colored, spreading.
Aerial mycelium: White cottony tufts all over surface.
Soluble pigment: None, purplish pigment developing only in 7 days.
8. Starch plate, 25°, 12 days.
Growth: Spreading, central portion pink, with colorless to gray margin.
Aerial mycelium: Ash-gray powder all over surface of growth.
Enzymatic zone: 4-5 mm. wide.
9. Potato plug.
Growth: Glossy, yellow to red, with age, turning red-brown.
Aerial mycelium: None.
Color of plug: Unchanged.
10. Carrot, 25°, 22 days.
Growth: Abundant, raised, spreading, covering all plug, of a pinkish shade.
Aerial mycelium: Grayish, powdery, abundant, all over surface.
Color of plug: Unchanged.
11. Gelatin, 18°, 30 days.
Growth: Gray, moist, abundant surface growth.
Aerial mycelium: White, in patches.
Soluble pigment: Yellowish green, spreading into the insoluble portion.
Liquefaction: Medium, 2 cm. of gelatin in tube liquefied in 30 days.
12. Synthetic solution, 15 days.
Growth: Thin flakes throughout medium.
Aerial mycelium: None.
Soluble pigment: None.
When glycerin is substituted for saccharose, there is a heavy cream-colored pellicle formed, with greenish white aerial mycelium and greenish soluble pigment.
13. Milk, 37°.
Growth (25°): Faint, brownish growth on surface.
Coagulation: 6-15 days.
Peptonization: Begins soon after coagulation is complete, advances slowly, coagulum may not all be digested in 40 days, although with an early coagulation digestion is completed in 15-20 days.
Change of reaction: Faintly alkaline (1).
14. Glucose broth, 25°, 12 days.
Growth: Solid, cream-colored mass on surface of liquid, with pink tinge on reverse.
Aerial mycelium: Cream-colored, all over growth.
Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Excellent with all sources of carbon; it may be absent in some cultures.
2. *Proteolytic action*: Fair on milk.

3. *Change of reaction*: Usually to alkaline.
 4. *Inversion of sugar*: Positive.
 5. *Diastatic action*: Good, starch reduced in 14 days only to erythrodextrin (poor saccharogenic action); only fair diastatic action is obtained on the plate.
 6. *Growth on cellulose*: Scant, but definite.
- Hab.* Isolated from California and cranberry soils.

Actinomyces chromogenus strain 205

I. MORPHOLOGY.

1. Spirals.

Numerous, closed spirals, of the fist type produced on the different synthetic media; the side hyphae are often only of a wavy nature.

2. Conidia.

Oval to elliptical.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 15 days.

Growth: White, spreading deep into the medium.

Aerial mycelium: Ash-gray with brownish tinge.

Soluble pigment: None.

2. Calcium malate-glycerin agar, 15 days.

Growth: Spreading, colorless on and below surface of medium, edge myceloid.

Aerial mycelium: Abundant, cottony, all over growth; drops of water on surface; color of mycelium light grayish olive (Rdg. XLVI, 21'''-b), with narrow white margin turning in 30 days dark gray.

Soluble pigment: None.

3. Glucose agar, 15 days.

Growth: Abundant, chiefly on surface of medium, also in substratum; edge entire; color natal brown (Rdg. XL, 13'''-K), changing in 30 days to almost black.

Aerial mycelium: Abundant, cottony, covering all surface; color white with gray tinge; changing in 30 days to light gray.

Soluble pigment: Brownish pigment, is dissolving into medium.

4. Nutrient agar, 15 days.

Growth: Wrinkled, brown colored at first, later turning gray-green.

Aerial mycelium: White patches.

Soluble pigment: Brown.

5. Blood serum, 37°, 7 days.

Growth: Thin, spreading, brown smear.

Aerial mycelium: None.

Soluble pigment: Spreading, dark zone.

Liquefaction: None.

6. Egg-media, 37°.

Growth: Thin, spreading, gray growth, later becoming black (17 days).

Aerial mycelium: Dark gray.

Soluble pigment: Spreading black zone.

7. Potato plug, 25°, 12-15 days.

Growth: Small, wrinkled, black colonies.

Aerial mycelium: None.

Color of plug: All black.

8. Carrot, 15 days.
Growth: Abundant, spreading, cream-colored.
Aerial mycelium: White, cottony tufts all over surface; exuded drops are found on surface.
Color of plug: Dark-brown zone around growth.
9. Starch plate, 15 days.
Growth: Transparent, spreading.
Aerial mycelium: Buff-gray in concentric zones.
Enzymatic zone: 12-15 mm. wide.
10. Gelatin, 18°C.
Growth: Cream-colored, spreading on surface and side of tube; flaky in the medium.
Aerial mycelium: Abundant, white, cottony tufts covering all surface growth.
Soluble pigment: Dark brown, changing to deep olive-green, both in the liquefied and unliquefied portions.
Liquefaction: Rapid at first, later becoming very slow (5 mm. in 35 days) so that the liquefied portion may solidify again; the liquefaction is more rapid in presence of starch.
 This is the only organism of the whole group which exhibited the quinone action on gelatin; even this organism did not show it always.
11. Synthetic solution.
Growth: Thin pellicle on surface and colorless flakes throughout the medium.
Aerial mycelium: Smoke gray; when glycerin is substituted for saccharose, it is yellowish-buff.
Soluble pigment: None; when glycerin is present, in place of saccharose, it is deep yellow.
12. Milk, 37°. Soluble brown pigment produced.
Growth (25°): Dark brown ring.
Coagulation: None at 37°, a clot is often produced at 25°.
Hydrolysis: If no visible clot is obtained, the milk may undergo hydrolysis in 20 days, often no visible action is observed on the milk.
Change of reaction: Distinctly alkaline (3).
13. Glucose broth, 25°, 12 days.
Growth: Thin, brown ring on surface in contact with glass; some flaky colonies on bottom.
Aerial mycelium: None to grayish brown thin layer.
Soluble pigment: Dark brown all through liquid.
14. Utilization of different carbon compounds.

<i>Arabinose</i>	0	<i>Dextrose</i>	1-2	<i>Lactose</i>	2
<i>Glycerin</i>	1-2	<i>Saccharose</i>	1-3	<i>Maltose</i>	3-4
<i>Cellulose</i>	2	<i>Mannite</i>	1	<i>Starch</i>	4
<i>Organic acids</i>	2				

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Very weak, usually none or only faint traces with some sources of carbon.
2. *Proteolytic action:* Faint in milk; good on gelatin, both in presence and absence of 1 per cent starch.
3. *Change of reaction:* Usually none, with NaNO_3 as source of nitrogen; often faint acidity (glycerin as source of carbon) or slight alkalinity, in the case of vigorous growth; distinctly alkaline in acid gelatin in presence and absence of starch (P_H changed from 6.2 to 7.4 and 7.8); faintly acid in alkaline glucose broth.

4. *Inversion of sugar*: Positive or negative, the latter is obtained with weak growth.
 5. *Diastatic action*: Good on plate.
 6. *Production of tyrosinase*: This organism is the only one of the whole group, in addition to *A. scabies*, which produced a dark pigment on the tyrosin agar plate; the pigment was not as deep as that produced by *A. scabies*.
 7. *Growth on cellulose*: Very faint and only with certain methods; this organism does not attack cellulose readily.
- Hab.* Isolated by the writer and Curtis from Maine Aroostook soil.

Actinomyces citreus Krainsky 1914, p. 684, emend. Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

None or very few on synthetic agar and calcium malate agar; long narrow, open spirals on dextrose and starch agar, which are of the dextrorose type.

2. Conidia.

Synthetic agar: Spherical to oval, 1.2 to 1.8 x 1.2 to 1.5 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Abundant, spreading, chiefly on surface also to some extent into medium; raised, wrinkled; color citron-yellow (Rdg. XVI, 23-C).

Aerial mycelium: Covering all growth, same color (another strain has white aerial mycelium).

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Good, spreading growth, extending somewhat into the medium; cream-colored with yellowish tinge.

Aerial mycelium: Extensive, cottony, thick, covering all colony, except narrow edge; white, with mouse-gray tinge, later turning all light gray.

Soluble pigment: None.

3. Glucose agar.

Growth: Very extensive growth, chiefly on surface, penetrating into the medium only to a small extent; center much elevated, edge entire, glossy, color olive-yellow (Rdg. XXX, 23'').

Aerial mycelium: Cottony, in patches, covering only parts of growth; white colored; in 30 days aerial mycelium is found to be very abundant, thick and pinkish in color.

Soluble pigment: None.

4. Nutrient agar.

Growth: Restricted, cream-colored.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood agar, 37°.

Growth: Small, restricted, gray colonies.

Aerial mycelium: None.

Soluble pigment: None.

Hemolysis: Begins only late (6 days), advancing slowly; zone of hemolysis 0.5 mm. wide in 21 days.

6. Blood serum, 37°.

Growth: Minute cream-colored colonies.

Aerial mycelium: None.

Soluble pigment: None.

Liquefaction: None.

7. Egg-media, 37°.
Growth: Thin, spreading, wrinkled, cream-colored growth.
Aerial mycelium: None.
Soluble pigment: None.
8. Starch plate.
Growth: Abundant, yellowish-green color, characteristic.
Aerial mycelium: Abundant, pinkish colored.
Enzymatic zone: Fair, 5-8 mm. not perfectly cleared.
9. Potato plug.
Growth: Thin, wrinkled, gray-colored smear.
Aerial mycelium: Thin, white, but property lost with age, so that on later transfers none is observed.
Color of plug: Unchanged.
10. Carrot, 25°, 22 days.
Growth: Abundant, spreading, much folded, brownish colonies.
Aerial mycelium: Thin, powdery, white in rare patches.
Color of plug: Unchanged.
11. Gelatin.
Growth: Yellowish, restricted pellicle, floating on surface or falling to bottom of liquefied pit.
Aerial mycelium: White, scant.
Soluble pigment: None.
Liquefaction: Medium (1 cm. in 35 days).
12. Synthetic solution.
Growth: Small flakes on bottom.
Aerial mycelium: None.
Soluble pigment: None.
When glycerin is substituted for saccharose, there is formed a white surface pellicle, with a thin white aerial mycelium.
13. Milk, 37°.
Growth: Cream-colored surface zone and hydrolysis in 15 days at 25°.
Coagulation: 9-10 days.
Peptonization: Rapid; clot all digested in 20 days.
Hydrolysis: In certain tubes no coagulation takes place, but milk is slowly hydrolyzed.
Change of reaction: Distinctly alkaline (3).
14. Glucose broth, 25°, 12 days.
Growth: Thin, wide, yellowish ring on surface, in contact with glass; often an abundant surface pellicle is formed; few flakes on bottom and throughout medium.
Aerial mycelium: Scant white over edges of growth.
Soluble pigment: None to faint yellow.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: None or mere traces of nitrites accumulated, with moist carbon sources.
2. *Proteolytic action*: Very good in milk.
3. *Change of reaction*: Distinct alkalinity in milk; faint acidity in alkaline glucose broth.
4. *Inversion of sugar*: Positive.
5. *Diastatic action*: Fair on plate.
6. *Growth on cellulose*: None.

Hab. Isolated numerous times from Oregon adobe, New Jersey garden and orchard soils, and identified by Waksman and Curtis as belonging to the above species, although comparison with Krainsky's culture was impossible, as in the case of the other cultures that were believed to be the same as isolated by Krainsky.

Actinomyces diastaticus Krainsky 1914, p. 682, emend. Waksman and Curtis

It is not certain whether or not this organism is exactly the same as that described by Krainsky, since no comparison of cultures was made, it is related culturally and particularly in its diastatic action to the following two organisms or groups: *A. rutgersensis* and *A. lipmanii*; the *A. diastaticus* studied by Krainsky no doubt belongs to one of these three organisms or is at least a closely related form.

I. MORPHOLOGY.

1. Spirals.

Usually none on synthetic dextrose and calcium malate agar; sometimes fine, narrow, long spirals may be produced.

2. Conidia.

Synthetic agar: Oval shaped, 1.0 to 1.2 x 1.1 to 1.5 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Spreading, thin, gray, developing deep into medium.

Aerial mycelium: White, turning drab gray (Rdg. XLVI, 17^{'''}-d).

Soluble pigment: Brown to dark brown.

2. Calcium malate-glycerin agar.

Growth: Spreading on surface and deep into medium, edge glossy, entire; color brown.

Aerial mycelium: Thin, gray, somewhat powdery, covering whole surface except a narrow margin of colony left uncovered.

Soluble pigment: None.

3. Glucose agar.

Growth: Yellowish, spreading on surface in the form of minute colonies, running into one another, also deep into medium.

Aerial mycelium: None in 30 days.

Soluble pigment: None.

4. Nutrient agar.

Growth: Cream-colored.

Aerial mycelium: Thin, white.

Soluble pigment: None.

5. Blood agar, 37°.

Growth: Grayish-brown, restricted, folded.

Aerial mycelium: None.

Soluble pigment: Faint brown at first (2 days), later may disappear.

Hemolysis: Wide clear zone in 10-15 days.

6. Blood serum, 37°.

Growth: Restricted, gray colored, appearing late (12-15 days).

Aerial mycelium: None.

Soluble pigment: None.

Liquefaction: Started in 15 days.

7. Egg-media, 37°.

Growth: Very thin, spreading, wrinkled growth, cream-colored, with greenish tinge (5 days).
Aerial mycelium: None.
Soluble pigment: None.
8. Starch plate, 15 days.

Growth: Thin, spreading, colorless.
Aerial mycelium: None.
Enzymatic zone: Very broad, 20 mm., and more.
9. Potato plug.

Growth: Abundant, wrinkled, cream-colored, with greenish tinge, later (15 days) becoming brown.
Aerial mycelium: White, to gray; property is lost with age, so that, on continued cultivation, no aerial mycelium is produced.
Color of plug: Darkened at first; on continued cultivation no change of color of plug is produced.
10. Carrot, 25°, 22 days.

Growth: Abundant, spreading, folded, dark brown, covering all surface of plug.
Aerial mycelium: Scant, drab gray.
Color of plug: Brownish, all plug shrivelled up.
11. Gelatin, 18°.

Growth: Small cream-colored flakes dropping to bottom of liquefied portion; some growth on surface.
Aerial mycelium: Gray, covering all growth.
Soluble pigment: None.
Liquefaction: Rapid (3-4 cm. in 35 days).
12. Synthetic solution.

Growth: Flakes throughout medium, often a white surface pellicle is formed.
Aerial mycelium: None or some thin grayish layer.
Soluble pigment: None to yellow, often becoming brownish.
13. Milk, 37°.

Growth (25°): Brownish ring on surface.
Coagulation: 5-7 days.
Peptonization: Begins in 5-7 days, is completed in 25-30 days.
Change of reaction: Faintly alkaline (1).
14. Glucose broth, 25°, 12 days.

Growth: Ring of gray colonies on surface; round, colorless to grayish colonies on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.
15. Utilization of different carbon compounds.

Arabinose.....	4	Dextrose.....	4	Lactose.....	4
Glycerin.....	4	Saccharose.....	1-3	Maltose.....	4
Cellulose.....	0	Mannite.....	4	Starch.....	4
Organic acids.....	0-1				

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair in presence of most readily available sources of carbon; often none is evident, especially with poor growth.
2. *Proteolytic action:* Good in milk; very good on gelatin, but only faint when 1 per cent of starch is present.

3. *Change of reaction*: Distinct alkalinity, with sodium nitrate as a source of nitrogen; fairly alkaline in acid gelatin, but only faintly alkaline when 1 per cent of starch is present; faint alkalinity in milk; faint acidity in alkaline glucose broth.
 4. *Inversion of sugar*: None.
 5. *Diastatic action*: Excellent, all starch of a 1 per cent solution used up in 14 days; excellent on plate, zone more than 20 mm. wide in 15 days.
 6. *Growth in cellulose*: None or very scant.
- Hab.* California sandy loam.

Actinomyces 161

I. MORPHOLOGY.

This species resembles in many respects Krainsky's *A. erythrochromogenus*, but it does not produce the brown pigment characteristic of the *chromogenus* species on nutrient agar and gelatin.

Synthetic agar: Numerous open spirals formed as side branches of the main hyphae. The mycelium is fine, branching. No spirals are observed when saccharose is replaced by glycerin in the medium.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Spreading with irregular margin, developing deep into the medium; color at first white, later turning yellowish; agar around growth has a white milky surface.

Aerial mycelium: Thick, solid, white, all over growth and appears early.

Soluble pigment: Pomegranate purple (Rdg. XII, 71-i), later turning Bordeaux (Rdg. XII, 71-k) color. On repeated transfers, pigment becomes vinaceous colored.

2. Calcium malate-glycerin agar.

Growth: Restricted, thin on surface, developing well into the medium; surface smooth, edge lobose; color creamy with shade of pink.

Aerial mycelium: Thin, covering entire surface, white colored.

Soluble pigment: None.

3. Glucose agar.

Growth: Abundant, spreading, cream-colored, later turning brown chiefly on surface; center raised; surface presents cracking appearance, edge lobose.

Aerial mycelium: Thin, white, over entire surface (very thin over margin).

Soluble pigment: None. Faint brown in 20 days.

4. Nutrient agar.

Growth: Cream-colored.

Aerial mycelium: Few white patches.

Soluble pigment: None.

5. Blood serum, 25°, 30 days.

Growth: Restricted, gray colored.

Aerial mycelium: None.

Soluble pigment: None.

Liquefaction: None.

6. Egg-media, 25°, 30 days.

Growth: Spreading, cream-colored, with pinkish tinge.

Aerial mycelium: Cottony, white tufts all over surface, with pinkish tinge showing through.

Soluble pigment: Pinkish tinge.

7. Potato plug.
Growth: Wrinkled, cream-colored, becoming yellowish with age (30 days).
Aerial mycelium: White patches.
Color of plug: Purplish in 18 days.
8. Carrot, 25°, 22 days.
Growth: Restricted, later spreading, much raised, folded brownish colored.
Aerial mycelium: White, powdery, with shade of pink; center dark.
Color of plug: Dark brown.
9. Starch plate, 25°, 12 days.
Growth: Cream-colored, round colonies, with faint greenish tinge.
Aerial mycelium: White, granular in patches over surface.
Soluble pigment: None.
Enzymatic zone: 11-13 mm. wide.
10. Gelatin, 18°, 30-35 days.
Growth: Abundant, dense gray with pinkish tinge, chiefly on surface of liquefied portion.
Aerial mycelium: Abundant, white, powdery on surface of growth.
Soluble pigment: None.
Liquefaction: Slow, (0.5 cm. in 35 days) in presence of starch, more rapid in its absence.
11. Synthetic solution.
Growth: Greenish-brown colonies on surface with solid ring in contact with glass of tube.
Aerial mycelium: Usually none or scant white.
Soluble pigment: Greenish-brown to red brown.
12. Milk, 37°.
Growth (25°): Yellowish surface zone.
Coagulation: Begins in 7-8 days, advances very slowly and is not completed in 50 days.
Change of reaction: Faintly (1) to distinctly alkaline (2).
13. Glucose broth, 25°, 12 days.
Growth: Abundant, cream-colored growth on surface.
Aerial mycelium: Thin, powdery, rare, white, in tufts.
Soluble pigment: Dark brown, dissolving downward; often none, particularly if growth is limited.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Only with starch as source of carbon, not with saccharose or glycerin.
 2. *Proteolytic action:* Good in milk; excellent on gelatin, but only good in the presence of a 1 per cent starch.
 3. *Change of reaction:* Faintly alkaline in alkaline glucose broth; alkaline in solutions containing NaNO_3 as source of nitrogen and different sources of carbon; distinctly alkaline in acid gelatin, both in presence and absence of starch (from pH 6.2 changed to 7.6); distinctly alkaline in milk.
 4. *Inversion of sugar:* Positive.
 5. *Diastatic action:* Very good; all starch reduced in a 1 per cent solution in 14 days; good on plate.
 6. *Growth on cellulose:* None.
- Hab.* Isolated from California and Hawaiian soils (161).

Actinomyces exfoliatus Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Usually none or only a very faint wavy effect of the mycelium (synthetic agar); on some media, such as cellulose agar and dextrose agar, there is a distinct tendency to form spirals.

2. Conidia.

Synthetic agar: Oval, 1.0 to 1.5 x 1.2 to 1.8 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Colorless, later becoming brown with smooth glossy surface, developing deep into the medium; surface growth has at first a tendency to crack and peel off, but on successive cultivation this property is lost.

Aerial mycelium: White patches, soon covering all surface.

Soluble pigment: Brown, increasing on successive cultivation.

2. Calcium malate-glycerin agar, 15 days.

Growth: Restricted, cream-colored surface growth, penetrating to a small extent into medium; surface dry, edge myceloid.

Aerial mycelium: Very thin, powdery, white.

Soluble pigment: None.

3. Glucose agar, 15 days.

Growth: Folded, spreading both on surface and into medium, elevated in center, wrinkled, cream-colored with brownish tinge, becoming in 30 days all brown.

Aerial mycelium: None at first, white patches appear in 30 days.

Soluble pigment: None.

4. Nutrient agar.

Growth: Clear, developing deep into medium, none on surface.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood serum, 37°.

Growth: Glossy, restricted, cream-colored smear.

Aerial mycelium: None.

Soluble pigment: None.

Liquefaction: None.

6. Egg-media, 37°.

Growth: Minute yellowish colonies (5 days), remaining unchanged on continued incubation.

Aerial mycelium: None.

Soluble pigment: None.

7. Starch plate, 15 days.

Growth: Restricted, gray colored, changing to brown.

Aerial mycelium: Light buff-gray.

Enzymatic zone: Fair, 6-8 mm. wide, incompletely cleared.

8. Potato plug.

Growth: Good, somewhat wrinkled, at first gray, later brown colored.

Aerial mycelium: None.

Color of plug: Unchanged.

9. Carrot, 25°, 22 days.

Growth: Thin, cream-colored, restricted, net-like.

Aerial mycelium: None.

Color of plug: Unchanged.

10. Gelatin, 18°.
Growth: Cream-colored, flaky on bottom of liquefied pit.
Aerial mycelium: White or none.
Soluble pigment: None.
Liquefaction: Faint to fair.
11. Synthetic solution.
Growth: Minute colonies through medium.
Aerial mycelium: White, often none at all.
Soluble pigment: Yellow to light brown.
12. Milk, 37°.
Growth (28°): Cream-colored ring on surface.
Coagulation: Usually none, often a soft clot is produced in 8-12 days.
Peptonization: Slow, not clearing as perfectly as in the case of hydrolysis.
Hydrolysis: When the milk does not clot, it hydrolyzes very rapidly and is completed in 8-10 days.
Change of reaction: Strongly alkaline (4).
13. Glucose broth, 25°, 12 days.
Growth: Round, colorless, small colonies on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.
14. Utilization of different carbon compounds.
- | | | | | | |
|--------------------|----------------|-----------------|-----|--------------|---|
| Arabinose..... | 4 | Dextrose..... | 4 | Lactose..... | 4 |
| Glycerin..... | 3 | Saccharose..... | 3-5 | Malate..... | 3 |
| Cellulose..... | 1-3 | Mannite..... | 3 | Starch..... | 4 |
| Organic acids..... | 1-2 (tartrate) | | | | |

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Good with many sources of carbon, often (saccharose and glycerin) only traces are produced.
 2. *Proteolytic action*: Fair in milk; faint on gelatin, both in presence and absence of starch.
 3. *Change of reaction*: Distinctly alkaline, with different sources of carbon with NaNO_3 as source of nitrogen; slightly acid in acid gelatin, changing from pH 6.2 to pH 5.8.
 4. *Inversion of sugar*: Positive.
 5. *Diastatic action*: Very good, 1 per cent starch all used up in 14 days; fair on plate.
 6. *Growth on cellulose*: Good in solution and on plate; clear zone was formed on plate.
- Hab.* California upland and adobe soil.

Actinomyces flavus Krainsky 1914, p. 685, emend. Waksman and Curtis

I. MORPHOLOGY.

1. *Spirals*.
 Usually none; very coarse straight branching hyphae on synthetic media; some open spirals may be produced.
2. *Conidia*.
 Conidia are produced by beading effect and also hyphae breaking up into oval-shaped spores.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.
Growth: Colonies round, yellow to sulfur-yellow.
Aerial mycelium: Straw-yellow.
Soluble pigment: None.
2. Calcium malate-glycerin agar.
Growth: Spreading both on and below surface, edge myceloid; color between straw yellow and amber yellow (Rdg. XVI, 21').
Aerial mycelium: Thin, white, powdery over center of growth, leaving wide margin uncovered, appears in 3-4 days.
Soluble pigment: None.
3. Glucose agar.
Growth: Restricted, chiefly on surface of medium, center raised and folded; sulfur-yellow with brown shade in raised center.
Aerial mycelium: None at first, scant white to grayish later.
Soluble pigment: None.
4. Nutrient agar.
Growth: Gray, folded, spreading.
Aerial mycelium: None.
Soluble pigment: Faint brown.
5. Blood serum, 25°, 20 days.
Growth: Spreading, cream-colored.
Aerial mycelium: Scant, white, in patches.
Soluble pigment: Brown.
Liquefaction: None.
6. Egg-media, 25°, 20 days.
Growth: Abundant, much wrinkled, cream-colored, later becoming yellow.
Aerial mycelium: At first traces of white patches which do not develop further.
Soluble pigment: Purplish, developing only in 7 days.
7. Starch plate, 25°, 12 days.
Growth: Spreading, cream-colored, with pink tinge.
Aerial mycelium: White tufts, with shade of pink.
Soluble pigment: None.
Enzymatic zone: 10-11 mm. wide.
8. Potato plug.
Growth: Elevated, much wrinkled (barnacle-like), greenish-olive (characteristic).
Aerial mycelium: Thin, white.
Color of plug: Dark zone around growth.
9. Carrot, 25°, 22 days.
Growth: Abundant, raised, restricted, yellowish.
Aerial mycelium: Thin, sulfur-yellow layer.
Color of plug: Unchanged.
10. Gelatin, 18°, 35 days.
Growth: Heavy, colorless pellicle on bottom of liquefied portion; small yellowish masses on surface (in contact with glass).
Aerial mycelium: None.
Soluble pigment: Dull-brown only in liquefied portion.
Liquefaction: Rapid (3 cm. in 35 days).
11. Synthetic solution.
Growth: Yellowish, round, flaky colonies throughout the medium.
Aerial mycelium: White to straw-yellow.
Soluble pigment: None.

12. Milk, 37°. Dark brown soluble pigment.
Coagulation: 5-6 days.
Peptonization: Complete in 10-15 days.
Change of reaction: Distinctly alkaline (4).
13. Glucose broth, 25°, 12 days.
Growth: Small, white, radiating round colonies on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Mere traces of nitrites are accumulated, with most carbon compounds.
 2. *Proteolytic action*: Good on gelatin and milk.
 3. *Change of reaction*: Unchanged in gelatin (faintly acid in presence of starch); very acid on glucose broth (P_H changed from 7.9 to 5.2 in 15 days); distinct alkalinity in milk.
 4. *Inversion of sugar*: Positive.
 5. *Diastatic action*: Fair to good; zone on plate 10-11 mm. wide in 12 days at 25°.
 6. *Growth in cellulose*: Fair, with paper in solution as the only source of carbon.
- Hab.* Received from Dr. C. B. Lipman, who isolated it from the forming soil of Tortugas Island; isolated also from upland and adobe California soils.

Actinomyces 128

I. MORPHOLOGY.

1. *Spirals*.
Usually none on all the media studied. Under the microscope, the growth is found to consist of a large mass of minute tufts; the hyphae are coarse, straight, short, relatively unbranched, beaded; open spirals may be produced in certain instances.
2. *Conidia*.
Synthetic agar: Spherical, oval to rod-shaped, 0.75 to 1.0 x 1.0 to 1.5 μ .

II. CULTURAL CHARACTERISTICS.

1. *Synthetic agar*.
Growth: Spreading deep into the substratum, yellowish with greenish tinge.
Aerial mycelium: Powdery, covering all surface with distinct zone formation; Hathi gray (Rdg. LII, 38''''-b) color.
Soluble pigment: Greenish yellow.
2. *Calcium malate-glycerin agar*.
Growth: Spreading deep into the medium; surface limited only to aerial mycelium; color of growth primuline yellow (Rdg. XVI, 19').
Aerial mycelium: Powdery, covering center of growth leaving wide uncovered margin; pale mouse gray (Rdg. LI, 15''''') mixed with pale buff.
Soluble pigment: None.
3. *Glucose agar*.
Growth: Restricted, developing only to a very small extent into the medium; edge entire, color yellow with shade of dark, in 30 days turning black.
Aerial mycelium: Covering all surface, except a narrow margin; color light gull gray (Rdg. LIII, C. G. -9); margin pale buff.
Soluble pigment: Golden yellow throughout slant.

4. Nutrient agar.
Growth: Yellowish; the reverse is dark in center, with a yellowish zone and outer white zone.
Aerial mycelium: Abundant, mouse-gray, all over growth.
Soluble pigment: None.
5. Blood agar, 37°.
Growth: Green, spreading.
Aerial mycelium: White.
Soluble pigment: None.
Hemolysis: Excellent.
6. Blood serum, 37°.
Growth: Thin, spreading, gray smear in 4 days, with glossy surface.
Aerial mycelium: White, developing at an early date (4 days).
Soluble pigment: None.
Liquefaction: Rapid in 7-8 days.
7. Egg-media, 37°.
Growth: Round, yellow colonies.
Aerial mycelium: Abundant, white, all over growth.
Soluble pigment: None.
8. Starch plate, 25°, 15 days.
Growth: Greenish-yellow, spreading growth, developing deep into the medium.
Aerial mycelium: Gray with tinge of yellow.
Enzymatic zone: Good (12-15 mm. wide).
9. Potato plug.
Growth: Sulfur-yellow (4 days), wrinkled.
Aerial mycelium: Abundant ash-gray, all over growth, appears only in 6-7 days.
Color of plug: Narrow black zone around growth, non-spreading.
10. Carrot, 25°, 22 days.
Growth: Abundant, entire, spreading, yellowish with greenish tinge.
Aerial mycelium: Membranous, all over surface of growth; white, with yellow shade.
Color of plug: Unchanged.
11. Gelatin, 18°.
Growth: Yellowish-green surface pellicle consisting of a mass of small colonies; there is also a mass of flakes on bottom of liquefied portion.
Aerial mycelium: Abundant, white, cottony.
Soluble pigment: None.
12. Synthetic solution, 25°, 15 days.
Growth: Small, white colonies throughout medium (attached to glass of tube) and on surface.
Aerial mycelium: Ash-gray.
Soluble pigment: Greenish-yellow.
13. Milk, 37°.
Growth (25°): Cream-colored to brownish surface ring.
Coagulation: 3-6 days.
Peptonization: Begins soon after coagulation, advances rapidly and is all completed in 15-30 days.
Change of reaction: Faintly alkaline (1).
14. Glucose broth, 25°, 12 days.
Growth: Thick, sulfur-yellow mass on surface, chiefly in contact with glass of tube.
Aerial mycelium: Cottony, white.
Soluble pigment: None.

15. Utilization of different carbon-compounds.

<i>Arabinose</i>	0	<i>Dextrose</i>	2	<i>Lactose</i>	2
<i>Glycerin</i>	1	<i>Saccharose</i>	1	<i>Maltose</i>	3
<i>Cellulose</i>	1-2			<i>Starch</i>	3
<i>Organic acids</i>	1-2				

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Usually none or mere traces (maltose and glycerin).
 2. *Proteolytic action*: Very good on milk; good on gelatin, both in presence and absence of starch.
 3. *Change of reaction*: No change, slight acidity or alkalinity with NaNO_3 , depending on source of carbon; faintly alkaline in milk; distinctly alkaline in glucose broth and in gelatin, both in presence and absence of starch.
 4. *Inversion of sugar*: None.
 5. *Diastatic action*: Fair, starch not used up in a 1 per cent solution in 20 days; good on plate, zone 12-15 mm. wide.
 6. *Growth on cellulose*: None or very scant.
- Hab.* Oregon adobe soil (128).

Actinomyces fradii Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

None observed on most media; only straight, branching hyphae; on glycerin synthetic agar some spiral formation is observed which is of a dextrorose type.

2. Conidia.

Synthetic agar: Rod to oval-shaped, 0.5×0.75 to 1.25μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Smooth, spreading, colorless, developing deep into the medium; often a pale yellow orange (Rdg. III, 15-f) shade in reverse.

Aerial mycelium: Thick cottony mass soon covering all surface, of a sea-shell pink color (Rdg. XIV, 11-f).

Soluble pigment: None.

2. Calcium malate-glycerin agar, 15 days.

Growth: Spreading, orange-colored, growing deep into medium.

Aerial mycelium: Cottony patches at first, in 25 days, mycelium covers all surface; color of mycelium is the same as on No. 1, but with lighter colored edge.

Soluble pigment: None.

3. Glucose agar.

Growth: Restricted glossy growth on surface of medium; surface of growth forming a net-work in places; edge lichnoid; color cartridge buff (Rdg. XXX, 19'-f).

Aerial mycelium: None in 15 days, in 25 days patches of characteristic sea-shell pink color appears.

Soluble pigment: None.

4. Nutrient agar.

Growth: Yellowish, turning later (10 days) to orange-yellow, restricted.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood agar, 37°.
Growth: Good, brown, crumpled growth is obtained in 2 days, later (21 days) changing to reddish colored.
Aerial mycelium: None.
Soluble pigment: None.
Hemolysis: None.
6. Blood serum, 37°.
Growth: Orange-colored, restricted.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: None.
7. Egg-media, 37°.
Growth: Restricted, wrinkled, orange-colored growth.
Aerial mycelium: White patches.
Soluble pigment: None.
8. Starch plate.
Growth: Spreading, colorless.
Aerial mycelium: Of the characteristic sea-shell pink color.
Enzymatic zone: Broad, 12-15 mm.
9. Potato plug.
Growth: Restricted, orange-colored, characteristic.
Aerial mycelium: Thin, cream-colored patches.
Color of plug: Unchanged to faint brown.
10. Carrot, 25°, 22 days.
Growth: Orange-colored to brownish-orange, spreading, much wrinkled with lichnoid margin.
Aerial mycelium: None.
Color of plug: Unchanged.
11. Gelatin, 18°, 30 days.
Growth: Cream-colored to brownish dense growth on surface of liquefied portion.
Aerial mycelium: White or none.
Soluble pigment: None.
Liquefaction: Rapid, on continued cultivation it becomes slower.
12. Synthetic solution.
Growth: Minute colonies through medium and on surface.
Aerial mycelium: None.
Soluble pigment: None.
13. Milk, 37°.
Growth (25°): Faint, cream-colored surface ring.
Coagulation: 10-12 days.
Peptonisation: Begins as soon as coagulation is complete, proceeds rapidly and coagulum is all digested in 20 days.
Hydrolysis: In certain cases, no coagulum is formed, but milk is slowly hydrolyzed downward; clearing is not completed in 30 days.
Change of reaction: Fairly alkaline (2).
14. Glucose broth, 25°, 12 days.
Growth: Dense, narrow, orange-colored ring on surface in contact with glass; also abundant, colorless flaky mass on bottom.
Aerial mycelium: None.
Soluble pigment: None.
15. Utilization of different carbon compounds.

<i>Arabinose</i>	3	<i>Dextrose</i>	4	<i>Lactose</i>	3
<i>Glycerin</i>	3	<i>Saccharose</i>	1-2	<i>Maltose</i>	2
<i>Cellulose</i>	0	<i>Mannite</i>	3	<i>Starch</i>	4
<i>Organic acids</i>	0-1				

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Good with different sources of carbon; often only traces are produced.
2. *Proteolytic action*: Good in milk; fair on gelatin; although the liquefying power of the gelatin is very good to excellent, the hydrolysis of the gelatin is rather limited.
3. *Change of reaction*: Faint alkalinity for all sources of carbon used, with NaNO_2 as source of nitrogen; unchanged in gelatin, with slight acidity in gelatin in presence of starch; fair alkalinity in milk.
4. *Inversion of sugar*: None.
5. *Diastatic action*: Very good, all starch used up in 14 days; good on plates, diastatic zone 12-15 mm. wide in 15 days.
6. *Growth on cellulose*: None on cellulose in solution; fair on plates, but no clear zone formed.

Hab. Isolated from California adobe soils.

Actinomyces 96

I. MORPHOLOGY.

1. *Spirals*.
None on the media studied; only some curling found in side branches, although no regular spirals were formed.
2. *Conidia*.
Synthetic agar: Hyphae break up into spherical to oval-shaped spores.

II. CULTURAL CHARACTERISTICS.

1. *Synthetic agar*.
Growth: Colorless, thin, spreading, chiefly in the medium; surface growth limited almost entirely to the aerial mycelium.
Aerial mycelium: At first (4 days) forming gray zones, later becoming pallid neutral-gray (Rdg. LIII, N. G.-f); it covers the whole surface of growth as a thin powdery layer with distinct zone formation.
Soluble pigment: None.
2. *Calcium malate-glycerin agar*.
Growth: Thin, spreading, developing to some depth into the medium; edge entire; color brownish.
Aerial mycelium: Fine network covering growth in zones; light mouse-gray color (Rdg. LI, 15''''-b).
Soluble pigment: Faint brownish.
3. *Glucose agar*.
Growth: Spreading both on surface and into the medium; center raised, color creamy, turning dark.
Aerial mycelium: Abundant, covering all growth, deep dull-gray (Rdg. LIII, C. G. -7), with wide outer white zone.
Soluble pigment: None.
4. *Nutrient agar*.
Growth: Brownish with smooth surface.
Aerial mycelium: Powdery, white with gray tinge, covering all the surface.
Soluble pigment: Brown.

5. Blood serum.
Growth: None.
6. Egg-media, 37°, 7 days.
Growth: Spreading, cream-colored.
Aerial mycelium: White cottony tufts all over surface of growth.
Soluble pigment: None.
7. Starch plate, 25°, 15 days.
Growth: Grayish brown, with dark ring.
Aerial mycelium: Gray.
Enzymatic zone: Fair (8-10 mm. wide), starch not perfectly cleared.
8. Potato plug.
Growth: Cream-colored at first (4 days), later becoming black (15 days), spreading rapidly and surrounding all the plug. The growth of this organism and of *A. 206* results in the destruction of the whole plug in 30 days.
Aerial mycelium: At first (4-5 days), thin white, later (15 days) becoming abundant, white colored with greenish tinge.
Color of plug: Brown.
9. Carrot, 25°, 22 days.
Growth: Abundant, enveloping all the surface of the plug.
Aerial mycelium: Abundant, ash-gray to purplish gray, powdery, all over surface.
Color of plug: Dark brown.
10. Gelatin, 18°, 35 days.
Growth: Spreading, yellowish, dropping in the form of flakes to bottom of liquefied portion.
Aerial mycelium: White, all over growth.
Soluble pigment: Faint yellowish in liquefied portion.
Liquefaction: Slow at first, then rapid, with nearly all tube-liquefied in 35 days.
11. Synthetic solution.
Growth: Scant flaky growth on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.
12. Milk, 37°.
Growth (25°): Abundant surface pellicle, pinkish colored with gray aerial mycelium.
Coagulation: 10-12 days.
Peptonisation: Begins soon after coagulation is complete, advances with a fair speed and in 30 days the clot is all digested.
Change of reaction: Fairly alkaline.
13. Glucose broth, 25°, 12 days.
Growth: Thick brown colored, wide ring on surface, chiefly in contact with glass.
Aerial mycelium: Abundant, ash-gray, cottony.
Soluble pigment: Faint brown or none.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Fair, with glycerin as a source of carbon.
2. *Proteolytic action*: Good on milk; very good on gelatin, but only good, in the presence of 1 per cent starch.
3. *Change of reaction*: Distinctly alkaline in milk and gelatin, both in presence and absence of starch; alkaline glucose broth is often turned acid.

4. *Inversion of sugar*: Negative.
 5. *Diastatic action*: Fair in plate, saccharogenic power much weaker than amylolytic.
 6. *Growth on cellulose*: None or scant.
- Hab.* A very common soil organism (96).

Actinomyces griseus

This organism was isolated numerous times from the soil.

The term *A. griseus* was used before by Krainsky (22, p. 682), so that the description of the latter is itself an emendation. Although this organism was originally (44) identified with the organism described by Krainsky, under the same name (from description only, without any actual comparison of cultures), this identification should be, therefore, corrected. The culture described here possesses a very strong proteolytic power, while Krainsky stated that his culture was not strong proteolytically.

I. MORPHOLOGY.

1. Spirals.

None on most media; on certain others, such as cellulose agar, spirals are readily formed. Drechsler (13) observed the proliferations of fertile branches at moderately close intervals along the axial hyphae, but no spirals were formed; he is perfectly right in the first, but not exactly in the second, since few closed spirals were formed by this organism on certain media.

2. Conidia.

Synthetic agar: Rod-shaped to short cylindrical, 0.8×0.8 to 1.2μ ($0.8 \times 0.8-1.5 \mu$).

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Thin, spreading, developing deep into the medium, at first colorless, then turning olive-buff (Rdg. XL, 2nd-d). This pigment may not be produced on successive transfers.

Aerial mycelium: Thick, powdery mass of a water-green color (Rdg. XLI, 25th-d).

Soluble pigment: None; reverse of growth brownish with age (24 days).

2. Calcium malate-glycerin agar.

Growth: Growth thin, spreading, penetrating deep into medium; color greenish yellow with dark shade.

Aerial mycelium: Thin, covering all colony except a narrow edge; color approaching tea-green (Rdg. XLVII, 25th-C).

Soluble pigment: None.

3. Glucose agar.

Growth: Elevated somewhat at center, radiating towards periphery; edge broken; cream-colored, with shade of orange.

Aerial mycelium: Appearing only in dried-up portion of growth in a fine powdery form; white colored, later turning cream-colored.

Soluble pigment: None.

4. Nutrient agar.
Growth: Abundant, cream-colored to almost transparent.
Aerial mycelium: Abundant (4 days); color characteristic (as on No. 1).
Soluble pigment: None.
5. Blood agar, 37°.
Growth: Extensive, greenish, wrinkled growth is obtained in 24 hours
Aerial mycelium: White with shade of the characteristic greenish color.
Soluble pigment: None, often faint brown.
Hemolysis: Excellent, zone 1 cm. wide in 4 days.
6. Blood serum, 37°.
Growth: Thin spreading, grayish colored, with glossy surface.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: Rapid, begins in 2-3 days.
7. Egg-media, 37°.
Growth: Spreading, wrinkled cream-colored growth, with greenish tinge.
Aerial mycelium: None.
Soluble pigment: None.
8. Starch plate, 15 days.
Growth: Thin, spreading, transparent.
Aerial mycelium: Gray.
Enzymatic zone: 12-15 mm.
9. Potato plug.
Growth: Much wrinkled, yellowish.
Aerial mycelium: Powdery, characteristically colored (as on No. 1), all over growth.
Color of plug: Brownish in upper portion.
10. Carrot.
Growth: Sulfur-yellow to dirty yellow, folded, spreading.
Aerial mycelium: Greenish-yellow, all over growth.
Color of plug: Unchanged.
11. Gelatin, 18°.
Growth: Greenish-yellow or cream-colored with brownish tinge, developing deep into the substratum.
Aerial mycelium: White-gray, with greenish tinge.
Soluble pigment: None.
Liquefaction: Rapid (3 cm. in 35 days).
12. Synthetic solution.
Growth: Flakes throughout medium.
Aerial mycelium: None.
Soluble pigment: None.
13. Milk, 37°.
Growth (25°): Cream-colored ring on surface.
Coagulation: Rapid (2-4 days) clot formation.
Peptonization: Rapid (3-4 days), clearing up all the milk.
Hydrolysis: Sometimes the tube is rapidly hydrolyzed, without previous coagulation, which is due to the strong proteolytic action of the organism.
Change of reaction: Most alkaline (4).
14. Glucose broth, 25°, 12 days.
Growth: Abundant mass over entire surface of liquid, of yellowish color with greenish tinge; much folded.
Aerial mycelium: Powdery, of the characteristic tea-green color.
Soluble pigment: Very faint brown begins to appear in upper portion of liquid.

15. Utilization of different carbon compounds.

<i>Arabinose</i>	3	<i>Dextrose</i>	4	<i>Lactose</i>	3
<i>Glycerin</i>	1	<i>Saccharose</i>	1-2	<i>Maltose</i>	4
<i>Cellulose</i>	0-1	<i>Mannite</i>	3	<i>Starch</i>	4
<i>Organic acids</i>	1-3 (malate).				

This is one of the very few species which use glycerin only to a very limited extent.

16. Utilization of different nitrogen compounds.

<i>Ammonium sulfate</i>	1-2	<i>Ammonium carbonate</i>	0
<i>Sodium nitrite</i>	1	<i>Acetamide</i>	1
<i>Sodium nitrate</i>	1-2	<i>Leucin</i>	3
<i>Glycocoll</i>	3-4	<i>Casein</i>	5
<i>Asparagin</i>	3	<i>Fibrin</i>	4
<i>Egg-albumin</i>	4	<i>Urea</i>	1
<i>Peptone</i>	5		

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Fair, in presence of starch, little or none in presence of saccharose or glycerin.
 2. *Proteolytic action*: The most active organism in the whole series on milk, gelatin and different proteins.
 3. *Change of reaction*: Distinct alkalinity for all sources of carbon used, with NaNO_3 as source of nitrogen; strongly alkaline in milk, glucose broth and gelatin, both in absence and presence of starch.
 4. *Inversion of sugar*: None.
 5. *Diastatic action*: Very good; 1 per cent starch disappears at the end of 14 days; good on plate.
 6. *Growth on cellulose*: Very scant to fair; good growth on sterile soil.
- Hab.* Isolated from Texas loam, Oregon and California adobe soils.

Actinomyces 218

An organism closely related to the culture previously described (*A. griseus*), but producing a brown pigment on protein containing media and not so strong proteolytically.

I. MORPHOLOGY.

1. *Spirals*.
None on most media; often a few short, open spirals are produced.
2. *Conidia*.
Oval-shaped, produced abundantly.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

- Growth*: Spreading, developing deep into the medium; cream-colored.
Aerial mycelium: Appears early and covers all surface; powdery, olive-buff to water-green color.
Soluble pigment: None.

2. Calcium malate-glycerin agar.
Growth: Thin, spreading, cream-colored, penetrating to some extent into medium; edge erose.
Aerial mycelium: Thin, netted layer all over growth, surface netted; of a water-green color (Rdg. XLI, 25'''-d).
Soluble pigment: None.
3. Glucose agar.
Growth: Restricted, limited to surface, edge entire; yellowish-brown.
Aerial mycelium: In patches all over growth; pale olive-buff (Rdg. XL, 21'''-f); covering all surface in 30 days.
Soluble pigment: None
4. Nutrient agar.
Growth: Glistening, cream-colored at first, later (24 days) becoming brown.
Aerial mycelium: Abundant, white, all over surface.
Soluble pigment: Brown.
5. Blood serum, 37°, 15 days.
Growth: Glossy, elevated, gray colonies.
Aerial mycelium: Abundant, cream-colored, often in tufts.
Soluble pigment: Spreading, brown, becoming dark with age.
Liquefaction: Slow, begins in 25 days.
6. Egg-media, 37°, 15 days.
Growth: Abundant, cream-colored, spreading, with brownish tinge.
Aerial mycelium: Abundant, characteristically (olive buff) colored.
Soluble pigment: Spreading purple.
7. Starch plate, 25°, 12 days.
Growth: Spreading, cream-colored, with yellowish tinge.
Aerial mycelium: White powder all over surface of growth.
Enzymatic zone: 10-12 mm. wide.
8. Potato plug.
Growth: Brownish
Aerial mycelium: White, turning olive-buff.
Color of plug: Unchanged at first, later (20 days) turning faintly brown.
10. Carrot, 25°, 22 days.
Growth: At first (7 days) very scant, later it develops into a spreading gray growth, with an entire edge and smooth surface.
Aerial mycelium: White with shade of tea-green.
Color of plug: Unchanged.
11. Gelatin, 18°, 30 days.
Growth: Deep growing, cream-colored turning brown, spreading, abundant.
Aerial mycelium: White in upper portion of growth.
Soluble pigment: Deep brown, spreading through liquefied portion.
Liquefaction: Slow in absence of carbohydrate; in presence of starch more rapid (1½ cm. in 35 days).
12. Synthetic solution, 15 days.
Growth: Scant, white surface pellicle.
Aerial mycelium: None.
Soluble pigment: None.
13. Milk, 37°.
Growth (25°): Brownish ring on surface; soluble brownish pigment.
Coagulation: None.
Hydrolysis: Rapid in 10-12 days.
Change of reaction: Fairly alkaline (2).

15. Glucose broth, 25°, 12 days.

Growth: Wide, yellowish ring on surface in contact with glass, flaky mass on bottom.

Aerial mycelium: Characteristic yellowish, on surface of upper portion of growth.

Soluble pigment: Faint brown.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair, with most sources of carbon.

2. *Proteolytic action:* Very good on gelatin, both in presence and absence of starch.

3. *Change of reaction:* Distinctly alkaline in acid gelatin both in presence and absence of starch, from P_H 6.2 to P_H 8.0 and 8.2.

4. *Inversion of sugar:* None.

5. *Diastatic action:* Good on plate.

6. *Growth on cellulose:* Fair to good.

Hab. Isolated from sewage of trickling filter (Plainfield, N. J.).

Actinomyces halstedii Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: Closed spirals born as branches of the hyphae, 7 to 10 μ in diameter. When the organism looses, on continued cultivation, its ability to produce the typical aerial mycelium, no spirals are found in microscopic studies.

2. Conidia.

Synthetic agar: Oval to rod-shaped, 1.0 to 1.2 \times 1.2 to 1.8 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Abundant, heavy, spreading, penetrating into the medium; surface smooth, much raised; color at first light, turning to dark and almost black.

Aerial mycelium: White, turning gull-gray (Rdg. LIII, C. G.) on successive transfers, the culture looses the property of producing aerial mycelium.

Soluble pigment: None; white milky crust on agar around growth.

2. Calcium malate-glycerin agar.

Growth: Thin, spreading growth penetrating deep into medium; edge entire glossy; color dark with transparent margin.

Aerial mycelium: Thin, covering only center of growth, leaving wide bare margin; aerial mycelium deep mouse gray (Rdg. 15^{''''''}-1).

Soluble pigment: None.

3. Glucose agar.

Growth: Spreading, extensive, lichnoid; center much elevated, edge wrinkled; colorless at first, with brown center, later becoming all dark brown.

Aerial mycelium: None.

Soluble pigment: None.

4. Nutrient agar.

Growth: Restricted, wrinkled, cream-colored.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood serum, 37°. No growth.
6. Egg-media, 37°. No growth, or restricted, round cream-colored patches.
7. Starch plate.
Growth: Abundant, brownish colored, surface glossy.
Aerial mycelium: None.
Enzymatic zone: Very broad (20 mm. and more).
8. Potato plug.
Growth: Abundant, wrinkled with moist surface, cream-colored with green tinge.
Aerial mycelium: White at first, later property lost.
Color of plug: At first turned black; on continued cultivation property lost, plug remaining unchanged.
9. Carrot, 25°, 22 days.
Growth: Abundant, spreading, raised, surface smooth, moist; color at first gray, later turning greenish to dark green.
Aerial mycelium: None.
Color of plug: Unchanged.
10. Gelatin, 18°C.
Growth: Small, cream-colored round masses, dropping to bottom of liquefied portion.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: Rapid (2-3 cm. in 35 days).
11. Synthetic solution.
Growth: Small colonies through medium.
Aerial mycelium: None.
Soluble pigment: None.
When glycerin is substituted for saccharose, there is formed an abundant flaky growth on bottom of tube.
12. Milk, 37°. *Growth:* Cream-colored surface ring accompanied by rapid hydrolysis at 25°. *Coagulation:* 10 days.
Peptonization: Slow, begins in 10 days, not completed in 50 days.
Change of reaction: Fairly alkaline (2).
13. Glucose broth, 25°, 12 days.
Growth: Small, round, colorless colonies on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrile formation:* Good with starch as source of carbon, not with saccharose or glycerin.
 2. *Proteolytic action:* Scant on milk, fair on gelatin and glucose broth.
 3. *Change of reaction:* Fair alkalinity in milk; unchanged or slightly acid (in presence of 1 per cent starch) in acid gelatin; faintly acid in alkaline glucose broth.
 4. *Inversion of sugar:* Positive.
 5. *Diastatic action:* Excellent; 1 per cent starch in solution disappeared in 7 days; zone on plate very good (4).
 6. *Growth on cellulose:* None.
- Hab.* New Jersey garden, orchard and meadow subsoils.

Actinomyces hominis Bostroem (4)

I. MORPHOLOGY.

1. Spirals.

None on the media studied; aerial mycelium consists only of straight branching hyphae; a few dextrorose spirals are observed when the saccharose of the medium is replaced by glycerin.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 25°, 15 days.

Growth: Thin, spreading deep into the medium; color white with shade of yellow; when culture gets older (24 days), the drying up portions turn brown.

Aerial mycelium: Appears late (15 days); white with olive tinge.

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Spreeding, yellowish, chiefly on surface of medium.

Aerial mycelium: Gray, with olive-green tinge, all over growth.

Soluble pigment: None.

3. Glucose agar.

Growth: Yellowish, spreading, chiefly on surface of medium; surface of growth is a fine net-work.

Aerial mycelium: None in 15 days, later thin, white in patches.

Soluble pigment: None.

4. Nutrient agar.

Growth: Yellowish.

Aerial mycelium: White.

Soluble pigment: None.

5. Blood agar, 37°.

Growth: Good, rapid growth develops in 24 hours.

Aerial mycelium: None.

Soluble pigment: None.

Hemolysis: Distinct in 4 days, 3-4 mm. zone in 15 days.

6. Blood serum, 37°.

Growth: Spreading, transparent, glossy growth.

Aerial mycelium: None.

Soluble pigment: None.

Liquefaction: Rapid (4-8 days).

7. Egg-media, 37°.

Growth: Spreading, much wrinkled, yellowish.

Aerial mycelium: None.

Soluble pigment: None.

8. Starch plate, 25°, 15 days.

Growth: Thin, spreading, transparent.

Aerial mycelium: None.

Enzymatic zone: Good, 12-16 mm. wide.

9. Potato plug.

Growth: Abundant, wrinkled, yellowish to orange (4 days), later becoming brown (8 days).

Color of plugs: Unchanged at first, later (17 days) turning brown.

Aerial mycelium: White patches (4 days), later thin white all over growth (8 days).

10. Carrot, 25°, 22 days.
Growth: At first (7 days), restricted, tawny-olive (Rdg. XXIX, 17"-i) later spreading, abundant, wrinkled.
Aerial mycelium: White, with olive-green shade.
Color of plug: Unchanged.
11. Gelatin, 18°, 30 days.
Growth: Abundant, cream-colored, spreading, chiefly on surface, with some flakes on bottom of liquefied portion.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: Rapid in the absence of starch, very slow in the presence of starch.
12. Synthetic solution.
Growth: None. When glycerin is substituted for saccharose there is formed a heavy yellowish pellicle on surface of liquid, with a white aerial mycelium.
13. Milk, 37°.
Growth (25°): Abundant, cream-colored surface growth.
Coagulation: 5-6 days; much slower at 25°.
Peptonization: Begins in 5-6 days, proceeds rapidly and is completed in 20 days.
Change of reaction: Distinctly alkaline (3).
14. Glucose broth, 25°, 12 days.
Growth: Wide, thick, orange-colored ring on surface, in contact with glass of tube.
Aerial mycelium: Yellowish, thin on upper portion of ring.
Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Variable, depending on the source of carbon; good to very good with lactose, glycerin and maltose; none with saccharose and cellulose and faint with salts of organic acids.
2. *Proteolytic action*: Very good on milk and gelatin, much less on the latter in presence of starch; good on glucose broth.
3. *Change of reaction*: Strongly alkaline, with NaNO_3 as a source of nitrogen and with different carbohydrates as sources of carbon, particularly is that noticed in the case of maltose, where the P_H increased by 1.4 in 15 days. Distinctly alkaline in acid gelatin and milk, very acid in alkaline glucose broth.
4. *Inversion of sugar*: Negative.
5. *Diastatic action*: Good on plate.
6. *Growth on cellulose*: A good growth was obtained on sterile soil, very scant growth on filter paper.

Hab. Received from Dr. K. F. Meyer; received from Foulerton, who isolated it from abscess of palm of hand in 1911.

Actinomyces lavendulae Waksman and Curtis

I. MORPHOLOGY.

The aerial mycelium is very coarse, branching.

1. Spirals.

Synthetic agar: Close spirals, 5 to 8 μ in diameter, coiled up to form fists.
Dextrose agar: Spirals formed readily on this as well as on other media; spirals and straight branches break up readily into spores.

2. Conidia.

Synthetic agar: Abundant, oval, 1.0 to 1.2 \times 1.6 to 2.0 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.
Growth: Thin, spreading, colorless, developing deep into the medium.
Aerial mycelium: Cottony in spots, at first white, later turning deep vinaceous-lavender (Rdg. XLIV, 65'''-d); on successive transfer, the aerial mycelium may not be produced at all or remains white.
Soluble pigment: None.
2. Calcium malate-glycerin agar.
Growth: Minute cream-colored colonies on surface.
Aerial mycelium: None in 15 days; in 25 days lavender colored white patches appear all over surface.
Soluble pigment: None.
3. Glucose agar.
Growth: Thick, yellowish, spreading growth, penetrating to some extent into the medium, edge myceloid.
Aerial mycelium: Thick, cottony, all over surface, white with lavender tinge.
Soluble pigment: None.
4. Nutrient agar.
Growth: Wrinkled, gray.
Aerial mycelium: None.
Soluble pigment: Brown.
5. Blood serum, 37°.
Growth: Small, gray colonies.
Aerial mycelium: None.
Soluble pigment: Brown, spreading.
Liquefaction: None.
6. Egg-media, 37°.
Growth: A fine cream-colored net-work on surface of medium.
Aerial mycelium: None.
Soluble pigment: None.
7. Starch plate, 25°, 15 days.
Growth: Non-spreading, glistening, transparent, developing deep into the medium.
Aerial mycelium: Lavender colored.
Enzymatic zone: Good (3).
8. Potato plug.
Growth: Thin, wrinkled smear, cream-colored to yellowish.
Aerial mycelium: None.
Color of plug: Turns black; on continued cultivation property of coloring plug may be nearly lost.
9. Carrot.
Growth: Abundant, raised, wrinkled, spreading, brownish colored.
Aerial mycelium: Powdery over most of surface of growth; white, with faint shade of lavender.
Color of plug: Unchanged.
10. Gelatin, 18°.
Growth: Creamy to brownish, restricted on surface, with granule-like growth on bottom of liquefied portion.
Aerial mycelium: None to small white patches in contact with wall of tube.
Soluble pigment: Brown or none at all.
Liquefaction: Slow.

11. Synthetic solution.

Growth: Small, colorless, radiating colonies attached to glass; mass of floating colonies on surface.

Aerial mycelium: Characteristic lavender; property lost on transfer.

Soluble pigment: None.

12. Milk, 37°. Soluble brown pigment.

Growth (25°): Cream-colored ring.

Coagulation: None.

Hydrolysis: 20–30 days; at 25° hydrolysis proceeds more rapidly (4–5 days) proceeding from surface downwards.

Change of reaction: Strongly alkaline (4).

13. Glucose broth, 25°, 12 days.

Growth: Abundant, flaky mass on bottom of tube.

Aerial mycelium: None.

Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Positive, with starch as source of carbon, negative with saccharose and glycerin.

2. *Proteolytic action*: Fair on milk, faint to fair on gelatin.

3. *Change of reaction*: Acid on gelatin, both in presence and absence of starch, from P_H 6.2 to P_H 5.6 and 5.8; alkaline in milk.

4. *Inversion of sugar*: Positive, often a negative reaction is obtained.

5. *Diastatic action*: Very good; starch disappeared in 14 days in a 1 per cent starch solution; very good on plate, enzymatic zone 12–15 mm. in 15 days.

6. *Growth on cellulose*: Scant although definite.

Hab. Isolated from New Jersey orchard, California and Oregon white land; also isolated by Drechsler (13).

Actinomyces lipmanii Waksman and Curtis

This organism, or rather group, is closely related to the *A. diastaticus* of the writer and that described by Krainsky, and *A. rutgersensis*, particularly in some cultural and biochemical characters (strong diastatic action); but it differs from the other two organisms in morphology and certain cultural characters, as can be readily seen from the following description.

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: None on the media studied; hyphae straight, branching, showing in places some curvature.

2. Conidia.

Synthetic agar: 0.8 to 1.1 \times 1.0 to 1.5 μ .

I. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Abundant, much raised, at first colorless, wrinkled, later becoming light brown.

Aerial mycelium: White, turning gray (Rdg. LIII, 6) with white margin and white patches.

Soluble pigment: None.

2. Calcium malate-glycerin agar, 25°, 12 days.
Growth: Small, hyaline at first, later becoming dark, spreading as a very thin layer on surface and to a limited extent into the medium.
Aerial mycelium: Appears early, covering all the growth; mouse-gray, with wide white margin.
Soluble pigment: None.
3. Glucose agar.
Growth: Light yellow, irregular, spreading growth, developing deep into the medium.
Aerial mycelium: None in 20 days.
Soluble pigment: None.
4. Nutrient agar.
Growth: Yellowish, glossy, radially wrinkled.
Aerial mycelium: None.
Soluble pigment: None; when glycerin is present a characteristic green color is produced.
5. Blood agar, 37°.
Growth: Much folded, abundant, of a characteristic dirty gray color, with shade of green.
Aerial mycelium: None.
Soluble pigment: None.
Hemolysis: None.
6. Blood serum, 37°.
Growth: Cream-colored to almost transparent, thin, spreading.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: Medium (12-15 days).
7. Starch plate, 25°, 15 days.
Growth: Transparent at first, later becoming dark.
Aerial mycelium: None.
Enzymatic zone: Good to very good (3).
8. Potato plug.
Growth: Abundant, wrinkled growth, cream-colored.
Aerial mycelium: White patches at first (4 days), later becoming gray and covering all surface.
Color of plug: Purplish.
9. Carrot, 25°, 22 days.
Growth: Abundant, spreading, folded, of a dirty-gray color.
Aerial mycelium: At first scant, later ash-gray covering all surface.
Color of plug: Unchanged.
10. Gelatin.
Growth: Cream-colored flaky growth falling to bottom of liquefied portion.
Aerial mycelium: White-gray.
Soluble pigment: None.
Liquefaction: Rapid to medium.
11. Synthetic solution.
Growth: Flakes throughout medium, settling to bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.

12. Milk, 37°.

Growth (25°): Cream-colored ring on surface.

Coagulation: 8-9 days; the clot is often at (25°) nothing more than a thickening of the milk.

Peptonization: Begins soon after all the milk is coagulated, proceeds with a fair speed and is completed in 20-30 days.

Change of reaction: Fairly alkaline (2).

13. Glucose broth, 25°, 12 days.

Growth: White ring on surface; abundant colorless flaky mass on bottom of tube.

Aerial mycelium: None.

Soluble pigment: None.

14. Utilization of different carbon compounds.

<i>Arabinose</i>	0	<i>Dextrose</i>	3	<i>Lactose</i>	3
<i>Glycerin</i>	3	<i>Saccharose</i>	3	<i>Starch</i>	3
<i>Cellulose</i>	0	<i>Mannite</i>	3		

Addition of glycerin to organic or inorganic solid media results in the production of a distinctly characteristic, abundant green-colored growth.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Good, produced with different sources of carbon.

2. *Proteolytic action*: Good in milk and on gelatin.

3. *Change of reaction*: Increase in alkalinity with different sources of carbon and NaNO₂ as a source of nitrogen.

4. *Inversion of sugar*: Positive.

5. *Diastatic action*: Very good; all starch used up in 14 days in 1 per cent starch solution; very good on plate, zone 12-15 mm. wide in 15 days.

6. *Growth on cellulose*: None, or very scant.

Hab. This is one of the most common groups of soil actinomycetes. Isolated from the New Jersey garden and orchard, Iowa, Louisiana, California, North Dakota, Hawaiian, Alaska, Texas and Oregon adobe soils.

Actinomyces 168

I. MORPHOLOGY.

1. Spirals.

Numerous, closed or open, broad spirals on all media.

2. Conidia.

Oval shaped to elliptical.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Spreading deep into the medium, light sulfur yellow, later turning cadmium yellow (Rdg. III, 17); on repeated transfers for 3 years, yellow color is lost, color of growth is nearly white.

Aerial mycelium: Appears early (2-3 days), thin, white, with ash-gray patches.

Soluble pigment: Empire yellow (Rdg. IV, 21); property lost in 2 years.

2. Calcium malate-glycerin agar.

Growth: Colorless, later becoming cream-colored, penetrating deep into the medium; surface growth limited to aerial mycelium; edge myceloid.

Aerial mycelium: Thin layer all over growth; mouse-gray with white margin.

Soluble pigment: None.

3. Glucose agar.
Growth: Restricted, both on surface and into the medium, surface much folded, raised.
Aerial mycelium: Thin, powdery, white layer over upper portion of slant (in tube); in 30 days it covers all growth, pale gray color with white margin.
Soluble pigment: Yellowish green.
4. Nutrient agar.
Growth: Glistening, wrinkled, white.
Aerial mycelium: Abundant, white all over growth.
Soluble pigment: None.
5. Blood agar, 37°.
Growth: Brownish.
Aerial mycelium: White.
Soluble pigment: None.
Hemolysis: Narrow zone.
6. Blood serum.
Growth: Thin, brownish smear in 4 days; often growth is restricted, compact, orange-colored.
Aerial mycelium: None at first, later (15 days) white over edge of growth.
Soluble pigment: None.
Liquefaction: Slow, begins in 15 days.
7. Egg-media, 25°.
Growth: Thin, spreading brownish growth, radially wrinkled (6 days) remaining unchanged.
Aerial mycelium: None.
Soluble pigment: None.
8. Potato plug.
Growth: Abundant, wrinkled, cream-colored in 7 days.
Aerial mycelium: White, all over growth (4 days), later (30 days) becoming powdery.
Color of plug: Unchanged at first, later (30 days) turning faint brown.
10. Starch plate.
Growth: White spreading.
Aerial mycelium: Light gray.
Enzymatic zone: Broad (15-18 mm. wide), not all the starch is perfectly cleared.
11. Gelatin, 18°, 30 days.
Growth: Abundant, yellowish, spreading pellicle.
Aerial mycelium: Abundant, white.
Soluble pigment: Coloration of gelatin golden to faint brown.
Liquefaction: Rapid (3 cm. in 35 days, in presence of starch, 1½-2 cm. in absence of starch) to medium.
12. Synthetic solution.
Growth: Heavy yellowish pellicle on surface.
Aerial mycelium: White, with gray tinge.
Soluble pigment: Yellow to deep yellow.
13. Milk, 37°.
Growth (25°): Sulfur-yellow surface ring, with yellow soluble pigment.
Coagulation: 5-8 days.
Peptonization: Begins in 6-8 days, advances rapidly and is completed in about 20 days.
Hydrolysis: May take place in certain cultures in place of coagulation.
Change of reaction: Faintly alkaline (1).

14. Glucose broth, 25°, 12 days.

Growth: Thin, yellow pellicle, the part immersed in the liquid having spongy appearance.

Aerial mycelium: Thin, white.

Soluble pigment: Golden.

15. Effect of different carbon compounds.

<i>Arabinose</i>	0	<i>Dextrose</i>	5	<i>Lactose</i>	3-4
<i>Saccharose</i>	2	<i>Maltose</i>	4	<i>Cellulose</i>	1-3
<i>Mannite</i>	5	<i>Starch</i>	3	<i>Organic acids</i>	1-3

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Excellent, with different sources of carbon.
 2. *Proteolytic action:* Fair in milk; fair to good on gelatin, both in presence and absence of starch.
 3. *Change of reaction:* None or little change with small amount of growth; with heavy growth, reaction becomes distinctly alkaline with NaNO_3 as source of nitrogen and different sources of carbon; strongly alkaline in acid gelatin, both in presence and absence of starch (pH changed from 6.2 to 8.0); faintly alkaline in alkaline glucose broth and milk.
 4. *Inversion of sugar:* None.
 5. *Diastatic action:* Excellent, all starch disappeared in a 1 per cent solution in 14 days; good on plate.
 6. *Growth on cellulose:* Scant to fair with all methods studied.
- Hab.* California fertilized soil (168).

Actinomyces madurae (Vincent (39)) Lehmann and Newmann

I. MORPHOLOGY.

1. Spirals.

Usually none on most of the media studied; only straight, branching hyphae are obtained; often a few open or closed spirals are found.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Cream-colored, spreading, chiefly below the surface of the medium.

Aerial mycelium: Thin, powdery, white, appearing early (4 days).

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Spreading, cream-colored, developing deep into the medium.

Aerial mycelium: Gray, all over growth.

Soluble pigment: None.

3. Glucose agar.

Growth: Thin, glistening, pinkish, spreading on surface and into the medium.

Aerial mycelium: None in 15 days, later white powdery zone over margin of growth.

Soluble pigment: None.

4. Nutrient agar.

Growth: Abundant, cream-colored, spreading growth.

Aerial mycelium: White, appears late (15-20 days).

Soluble pigment: None.

5. Blood agar, 37°, 15 days.
Growth: Dark brown, spreading, rapid growth, obtained in 2 days.
Aerial mycelium: None.
Soluble pigment: None.
Hemolysis: Sharply defined, transparent zone 2-4 mm. in width.
 6. Blood serum, 37°.
Growth: Glossy, round, yellowish colonies obtained in 4 days.
Aerial mycelium: Thin, white in 4 days.
Soluble pigment: None.
Liquefaction: Rapid (4-8 days).
 7. Egg-media, 37°, 15 days.
Growth: Thin, spreading, wrinkled yellowish growth.
Aerial mycelium: Thin white patches.
Soluble pigment: None.
 8. Starch plate, 25°, 15 days.
Growth: Thin, spreading transparent.
Aerial mycelium: None.
Enzymatic zone: Very good, 12-16 mm. wide.
 9. Potato plug, 25°.
Growth: Abundant, wrinkled, sinking into the plug, yellow colored.
Aerial mycelium: None at first, later (15 days) white to gray all over growth.
Color of plug: Unchanged at first, later (17 days) turning faint brown.
 10. Carrot, 25°, 22 days.
Growth: Scant, transparent, yellowish, later spreading.
Aerial mycelium: None or white-grayish patches.
Color of plug: Unchanged.
 11. Gelatin, 18°, 30 days.
Growth: At first cream-colored, turning to greenish flaky masses, dropping to bottom of liquefied portion; brownish in exposed portion.
Aerial mycelium: Scant white patches.
Soluble pigment: None.
Liquefaction: Rapid (2-3 cm. depth of liquefied gelatin in tube in 35 days).
 12. Synthetic solution.
Growth: None. When glycerin is substituted for saccharose, a few colorless flakes are formed on bottom of tube.
 13. Milk, 37°.
Growth (25°): Cream-colored ring; action on milk proteins slow at 25°.
Coagulation: 3-4 days at 37°, while at 25° only thickening is observed followed by slow digestion.
Peptonization: Begins in 3-4 days, proceeds rapidly and is all completed in 10-30 days. Rapidity of coagulation and peptonization is very variable, depending on mother culture, amount of inoculum, etc.
Change of reaction: Faintly (1) to distinctly (3) alkaline.
 14. Glucose broth, 25°, 12 days.
Growth: Abundant, flaky, colorless colonies on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.
 15. Utilization of different carbon compounds.
- | | | | | | |
|----------------|---|--------------------|-----|-----------------|---|
| Dextrose..... | 2 | Glycerin..... | 1 | Saccharose..... | 1 |
| Cellulose..... | 0 | Organic acids..... | 1-2 | | |
| | | (lactate) | | | |

III. BIOCHEMICAL FEATURES

An organism under this name received from Král, was described by Foulerton and Price (see 16) as weakly proteolytic and with no diastatic action. The organism studied here proved to be very strongly proteolytic and possessing good diastatic properties.

1. *Nitrite formation*: Very limited, none (lactate) to fair with other sources of carbon.
2. *Proteolytic action*: Excellent on milk, peptone and gelatin (less in presence of starch).
3. *Change of reaction*: Fairly alkaline with NaNO_3 as the only source of nitrogen and different sources of carbon; distinctly alkaline in acid gelatin, in absence of available carbohydrates (only faintly alkaline in presence of starch); faintly alkaline in alkaline glucose broth and milk.
4. *Inversion of sugar*: Negative.
5. *Diastatic action*: Very good on plate.
6. *Growth on cellulose*: Scant.

Hab. The culture was received from Dr. K. F. Meyer of the Hooper Foundation, San Francisco, Cal., who had it from the American Museum of Natural History, received from Parke Davis Co., Feb., 1911, (No. 05); received by them in May, 1902, from Král; also received by the writer, from Parke Davis Co. (No. 01136). The description given above was based on the first culture; the second strain made a weaker growth on synthetic media.

Actinomyces pheochromogenus Conn 1917, p. 16

This organism has been isolated by the writer and Curtis from the soil, and has been also isolated and described by Conn (10).

I. MORPHOLOGY.

1. Spirals.

Dextrose agar: Many spirals of the narrower type, open, elongated; the spirals are sinistrose.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: The growth is developing deep into the medium, is at first colorless, later becoming brown to almost black.

Aerial mycelium: Abundant, all over growth, white colored, with brownish shade.

Soluble pigment: Black.

2. Calcium malate-glycerin agar.

Growth: Thin, spreading, penetrating to some extent into the medium; edge erose; color yellowish with brown tinge.

Aerial mycelium: Thin, white, cottony, covering central portion of surface, leaving rather wide, bare margin.

Soluble pigment: Brown to dark brown.

3. Glucose agar.

Growth: Restricted, much folded, growing deep into medium; color brown to dark brown.

Aerial mycelium: White, covering only portions of surface, later spreading over all surface; in 35 days, dark color of growth makes it look quite dark.

Soluble pigment: Brown.

4. Nutrient agar.
Growth: Thin, cream-colored, turning gray.
Aerial mycelium: None.
Soluble pigment: Deep brown.
5. Blood agar, 15 days, 37°C.
Growth: Brown.
Aerial mycelium: None.
Soluble pigment: None.
Hemolysis: None.
6. Blood serum, 37°.
Growth: Spreading, brown, developing late.
Aerial mycelium: None.
Soluble pigment: Chocolate brown.
Liquefaction: Faint.
7. Egg-media, 37°.
Growth: Slow, dark brown.
Aerial mycelium: White, net-like all over surface of growth.
Soluble pigment: Purplish, turning dark brown.
8. Starch plate, 12 days, 25°.
Growth: Spreading, brownish becoming later dark brown.
Aerial mycelium: White cottony tufts scattered over surface.
Soluble pigment: None.
Enzymatic zone: 6-8 mm. wide.
9. Potato plug.
Growth: Brown to almost black, wrinkled growth in 4-5 days.
Aerial mycelium: White patches.
Color of plug: Dark to almost black zone around growth.
10. Carrot, 25°, 22 days.
Growth: Scant, dark brown, late, developing only after 15 days.
Aerial mycelium: Scant, white.
Color of plug: Black soluble pigment is spreading over plug even before any appreciable growth is obtained.
11. Gelatin, 18°.
Growth: Abundant surface growth, spreading, cream-colored, turning brown in exposed portions.
Aerial mycelium: None at first, later white aerial mycelium may be found.
Soluble pigment: Deep brown.
Liquefaction: Slow to medium.
12. Synthetic solution (glycerin in place of saccharose).
Growth: Brownish heavy pellicle on surface with a few flakes throughout the medium.
Aerial mycelium: Abundant, buff colored.
Soluble pigment: Brown, spreading.
13. Milk, 37°. Soluble brown pigment at the end of 30 days.
Growth (25°): Dark to almost black surface ring; soluble black pigment.
Coagulation: Late (15-20 days); the soft coagulum settles to the bottom.
Peptonization: Slow, whey clouded.
Hydrolysis: The milk may hydrolyze without previous coagulation, particularly at 25°.
Change of reaction: Faintly alkaline (1).

14. Glucose broth, 12 days, 25°.

Growth: Dense, wrinkled, wide ring on surface of liquid only in contact with glass.

Aerial mycelium: Thin, white.

Soluble pigment: Deep brown.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Very good with glycerin as a source of carbon.

2. *Proteolytic action:* Faint in milk; good on gelatin.

3. *Change of reaction:* Faintly alkaline in milk, often unchanged; distinctly alkaline in acid gelatin (from P_H 6.2 to 7.7 and 8.4); faintly alkaline in glucose broth.

4. *Inversion of sugar:* Positive.

5. *Diastatic action:* Fair; tested only on plate, zone 6-8 mm. wide in 12 days at 25°.

6. *Growth on cellulose:* Usually scant.

Hab. Isolated from New Jersey orchard soil; also obtained from Dr. H. J. Conn of the New York Agricultural Experiment Station, who described and named this organism.

Actinomyces poolensis Taubenhaus 1918, p. 446

I. MORPHOLOGY.

1. Mycelium.

A very fine branching mycelium is produced; spirals are usually not produced, only in certain instances, a fine wavy effect is observed or close spirals may be formed.

2. Conidia.

Oval to elliptical.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Thin, colorless, spreading, developing deep into the medium; when saccharose is replaced by dextrose or glycerin, growth becomes very abundant, with yellow on reverse.

Aerial mycelium: White with shade of gray appearing late.

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Thin, spreading, cream-colored chiefly below the surface; edge myceloid; surface smooth.

Aerial mycelium: Thin over entire surface, leaving narrow margin uncovered; color light mouse-gray (Rdg. LI, 15'''-b) with creamy edge.

Soluble pigment: None.

3. Glucose agar.

Growth: Abundant, light brown, chiefly on surface, but also to some extent below the surface; surface glossy; center raised; edge entire.

Aerial mycelium: Usually none; certain strains (214) may produce a cottony aerial mycelium of a pale olive-bluff color (Rdg. XL, 21'''-f).

Soluble pigment: None.

4. Nutrient agar.

Growth: Translucent, yellowish growth, the surface of which presents a fine network.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood agar, 37°, 7 days.
Growth: Green, often yellowish-green, finely netted.
Aerial mycelium: None.
Soluble pigment: None to faint dark.
Hemolysis: None.
 6. Blood serum, 37°, 7 days.
Growth: Thin, spreading, gray smear.
Aerial mycelium: None or scant white patches.
Soluble pigment: None.
Liquefaction: None or faint, appearing late (25 days).
 7. Egg-media, 37°, 7 days.
Growth: Thin, spreading, wrinkled, brownish colored.
Aerial mycelium: None.
Soluble pigment: Faint brown.
 8. Starch plate, 25°, 12 days.
Growth: Restricted, cream-colored.
Aerial mycelium: White, cottony tufts all over surface.
Enzymatic zone: 9-10 mm. wide.
 9. Potato plug.
Growth: Thin, reddish-brown, sinking into plug.
Aerial mycelium: None.
Color of plug: Purplish, appearing late (17 days).
 10. Carrot, 25°, 22 days.
Growth: Thin, restricted, finely folded, brownish colored smear.
Aerial mycelium: None.
Color of plug: Unchanged.
 11. Gelatin, 18°, 35 days.
Growth: Small brownish flakes at bottom of liquefied portion.
Aerial mycelium: None.
Soluble pigment: None or faint yellow.
Liquefaction: Rapid to medium; about 2 cm. of depth of gelatin in tube is liquefied in 35 days.
 12. Synthetic solution. Very scant growth on saccharose; when glycerin is substituted into the solution, the results are as follows:
Growth: Abundant, brownish flaky growth on bottom of tube.
Aerial mycelium: None.
Soluble pigment: Faint brownish.
 13. Milk, 37°.
Growth (25°, 20 days): Brownish ring on surface.
Coagulation: 4-5 days.
Peptonization: Begins in 4-5 days, advances very rapidly and is completed in 9-10 days.
Hydrolysis: The milk is often hydrolyzed, without any visible coagulation.
Change of reaction: Strongly alkaline (4).
 14. Glucose broth, 25°, 12 days.
Growth: Thin, brownish ring on surface, in contact with glass.
Aerial mycelium: None.
Soluble pigment: None.
 15. Utilization of different carbon compounds.
- | | | | | | |
|--------------------|-----|-----------------|---|--------------|---|
| Arabinose..... | 0 | Dextrose..... | 1 | Lactose..... | 2 |
| Glycerin..... | 4 | Saccharose..... | 1 | Maltose..... | 1 |
| Cellulose..... | 0-1 | Mannite..... | 1 | Starch..... | 3 |
| Organic acids..... | 0-1 | | | | |

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: None or only traces with certain sources of carbon; fair with glycerin.
2. *Proteolytic action*: Excellent on milk; very good on gelatin; fair on peptone.
3. *Change of reaction*: None or only faint alkalinity with some sources of carbon and with NaNO_3 as only source of nitrogen; distinctly alkaline in gelatin and glucose broth; strongly alkaline in milk.
4. *Inversion of sugar*: Negative.
5. *Diastatic action*: Fair, zone on plate 9–10 mm. wide in 12 days, height of tube above control 5 mm.
6. *Growth on cellulose*: None or very scant, with methods used.

Hab. Isolated by Dr. J. Taubenhaus from diseased sweet potato tubers and by the writer several times from the soil; a closely related organism (214) was received from Dr. C. B. Lipman, who isolated it from forming soil on Tortugas Island.

Actinomyces purpeochromogenus Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.
Synthetic agar: None.
Starch agar: Few, imperfect spirals observed.
2. Conidia.
Spherical spores, 0.75 to 1.0 in diameter.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.
Growth: Slow, restricted, chiefly on surface of medium, surface smooth, center raised, color at first gray, later becoming brown with purplish tinge; margin yellow.
Aerial mycelium: Produced late, brownish purple to black; this is present as a thin, dry surface layer over growth, which makes it hard to distinguish it from the latter.
Soluble pigment: Brown to dark brown.
2. Calcium malate-glycerin agar.
Growth: Spreading, chiefly on surface, gray colored in the medium, black on surface of medium, margin spreading.
Aerial mycelium: None.
Soluble pigment: None in 15 days.
3. Glucose agar.
Growth: Abundant, restricted, developing 2–3 mm. into medium, 1 mm. above medium; at first gray, turning brown to dark brown.
Aerial mycelium: Brown to dark-brown aerial mycelium covering surface of growth.
Soluble pigment: Faint dark, later turning dark brown.
4. Nutrient agar.
Growth: Gray to brownish, penetrating into medium; surface growth becomes dark brown to almost black.
Aerial mycelium: None.
Soluble pigment: Brown.
5. Blood serum, 37°. No growth.
6. Egg-media, 37° and 25°. No growth or limited, thin, cream-colored smear.

7. Starch plate, 16 days, 25°.
Growth: The growth consists of a mass of small, dark-brown, individual colonies.
Aerial mycelium: Deep purple, with a glossy surface.
Soluble pigment: None.
Enzymatic zone: 4-5 mm., reduction incomplete.
8. Potato plug.
Growth: Restricted orange to orange-red colonies in 3-4 days, turning dark red in 15 days.
Aerial mycelium: None.
Color of plug: Unchanged, becoming faintly brown with age.
9. Carrot. No growth.
10. Gelatin, 30 days, 18°.
Growth: Slow, brownish colored.
Aerial mycelium: None.
Soluble pigment: Brown.
Liquefaction: Slow.
11. Synthetic solution.
Growth: Scant flakes on bottom of flask.
Aerial mycelium: None.
Soluble pigment: None.
12. Milk, 37°.
Growth (25°): Dark brownish ring on surface, in contact with glass; pinkish flakes in milk; brownish pigment in liquefied portion.
Coagulation: 10 days.
Peptonization: Begins in 10-12 days, advances slowly and is not completed in 50 days. In certain cases the digestion may proceed more rapidly and is completed in 30 days.
Change of reaction: Faintly alkaline (1).
13. Glucose broth.
Growth: Few flakes on bottom of flask.
Aerial mycelium: None.
Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: None with different carbon sources.
 2. *Proteolytic action*: Fair in milk and gelatin.
 3. *Change of reaction*: Faintly alkaline in milk.
 4. *Inversion of sugar*: Fair.
 5. *Diastatic action*: Scant; 1 per cent starch is not used in more than 4 weeks; faint diastatic action upon plate.
 6. *Growth on cellulose*: Usually scant.
- Hab.* Isolated from California adobe soil.

Actinomyces reticuli Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: None. Whirl formation characteristic of this as well as of the following species; the difference between this and the following organism is that the branches of this species do not form secondary whirls. This species represents a distinct group of organisms, widely separated from the other actinomycetes; several strains have been isolated which are as distinct from the

original *reticuli* as different species can be from one another, but in the same time form with it one group, that distinguishes them from the other species. The characteristic feature of this group of organisms is the peculiar method of branching of the aerial mycelium. Instead of the straight or curled branches usually found in the *Actinomyces* species on this medium, the organisms of this group produce a whirl of branches from a common point; these whirls are formed at intervals on the main hyphae. Conidia have been demonstrated to be formed from these branches.

Dextrose agar: Whirl formation predominant. Also tendency to form spirals, which are of the sinistrose type.

2. Conidia.

Synthetic agar: Spherical, 1.0 to 1.4 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Colorless, with yellowish tinge on surface, later becoming brownish, spreading, chiefly deep in the medium; surface growth limited to aerial mycelium.

Aerial mycelium: Thin, white, cottony, appearing in 7-12 days, forming a fine net-work with large holes in net (about 0.5 mm.).

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Scanty, colorless, developing to some extent into the medium.

Aerial mycelium: Small round patches of a naphthalene yellow color (Rdg. XVI, 23'-f).

Soluble pigment: None.

3. Glucose agar.

Growth: Restricted, brownish, center much raised above surface, penetrating only to a small extent into the medium.

Aerial mycelium: Cottony, covering all growth, of a naphthalene yellow color.

Soluble pigment: Brownish, diffusing through the medium.

4. Nutrient agar.

Growth: Wrinkled, gray, later becoming brownish.

Aerial mycelium: None.

Soluble pigment: Brown, spreading.

5. Blood serum, 37°.

Growth: Restricted, gray colonies.

Aerial mycelium: None.

Soluble pigment: Brown, spreading.

Liquefaction: None.

6. Egg-media, 37°.

Growth: Thin, spreading, radially wrinkled, gray.

Aerial mycelium: Gray with dark center.

Soluble pigment: Purple zone around growth.

7. Starch plate, 15 days.

Growth: Brownish gray.

Aerial mycelium: Lavender colored.

Enzymatic zone: Narrow, 4-5 mm. wide.

8. Potato plug.

Growth: Medium, gray with black center.

Aerial mycelium: Ash-gray.

Color of plug: Black.

9. Carrot, 25°, 22 days.
Growth: Abundant, yellowish colored, spreading, surface smooth, dry.
Aerial mycelium: None.
Color of plug: Unchanged.
10. Gelatin, 18°, 35 days.
Growth: Small, flaky, gray turning to brown, sinking to the bottom of the liquefied gelatin.
Aerial mycelium: Thin, white patches.
Soluble pigment: Faint to dark brown.
Liquefaction: Medium (1 cm. in 35 days) to slow.
11. Synthetic solution.
Growth: Small white flakes on bottom of tube, with abundant gray growth on surface.
Aerial mycelium: White.
Soluble pigment: None.
12. Milk, 3°. Soluble brown pigment.
Growth: Dark ring on surface, with no effect upon milk (only darkening) at 25° in 20 days.
Coagulation: 4-5 days.
Peptonization: Begins as soon as coagulation is complete, advances very slowly so that not all the coagulum is digested in 50 days.
Change of reaction: Unchanged.
13. Glucose broth, 25°, 12 days.
Growth: Large-sized colonies on bottom of tube.
Aerial mycelium: None.
Soluble pigment: Brown.
14. Utilization of different carbon compounds.
- | | | | |
|---------------------------|-----|-------------------------|---|
| <i>Dextrose</i> | 4 | <i>Saccharose</i> | 1 |
| <i>Cellulose</i> | 3 | <i>Maltose</i> | 3 |
| <i>Organic acid</i> | 1-2 | | |
| (acetate) | | | |
15. Utilization of different nitrogen compounds.
- | | | | |
|-------------------------------|---|---------------------------------|---|
| <i>Ammonium sulfate</i> | 0 | <i>Peptone</i> | 4 |
| <i>Sodium nitrite</i> | 1 | <i>Ammonium carbonate</i> | 0 |
| <i>Sodium nitrate</i> | 1 | <i>Acetamide</i> | 0 |
| <i>Glycocoll</i> | 3 | <i>Leucin</i> | 3 |
| <i>Asparagin</i> | 3 | <i>Fibrin</i> | 4 |
| <i>Egg-albumin</i> | 1 | <i>Urea</i> | 0 |

III. BIOCHEMICAL FEATURES.

- Nitrite formation*: Fair with different sources of carbon.
 - Proteolytic action*: Fair in milk, good on gelatin, very good on fibrin, good on peptone, faint on egg-albumin.
 - Change of reaction*: Unchanged in milk, unchanged or faint acidity with most proteins and amino acids, with glycerin as source of energy; faint acidity in alkaline glucose broth; fairly alkaline in acid gelatin.
 - Inversion of sugar*: Positive.
 - Diastatic action*: Fair, a 1 per cent starch solution not used up in 20 days; no reducing sugars found; faint to fair in plate, zone only 4-5 mm. wide in 15 days.
 - Growth on cellulose*: Scant.
- Hab.* Isolated from Iowa, California upland and adobe soils.

Actinomyces reticulys-ruber n. sp.

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: None. Whirl formation, both primary and secondary; primary whirls consisting of fewer branches, hyphae thicken at place of whirl formation.

Dextrose agar: Tendency to form spirals predominant. No spirals on calcium malate agar.

2. Conidia.

Synthetic agar: None observed (?).

Dextrose agar: Oval shaped.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 15 days.

Growth: Abundant, spreading, chiefly deep into the medium, where it is colorless; surface growth usually pink, often colorless.

Aerial mycelium: Thin, rose to pink colored, leaving a wide uncovered margin.

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Spreading on surface and deep into the medium; edge erose; growth colorless in the medium, red to rose-red on surface.

Aerial mycelium: Covering nearly all surface in a thin layer, white with rose pink shade.

Soluble pigment: None.

3. Glucose agar.

Growth: Extensive, spreading, penetrating deep into the medium; edge entire; color of surface growth rose-red (Rdg. XII, 71).

Aerial mycelium: Cottony, covering all surface; color white with shade of pink due to color of growth.

Soluble pigment: None.

4. Nutrient agar.

Growth: At first (4 days) red with yellowish margin, later becoming Acajou red (Rdg. XIII, 1'-i).

Aerial mycelium: None.

Soluble pigment: Brown.

5. Blood serum, 37°.

Growth: Glossy, grayish colonies appear early (4 days) and remain nearly unchanged.

Aerial mycelium: None.

Soluble pigment: Spreading, brown.

Liquefaction: None.

6. Egg-media, 37°, 15 days.

Growth: Spreading, wrinkled, brownish red in color, characteristic.

Aerial mycelium: Thin, white, with pink shade due to red color of growth.

Soluble pigment: Rapidly spreading, soluble black pigment, penetrating in 15 days through all the slant.

7. Starch plate, 25°, 15 days.

Growth: White with red tinge.

Aerial mycelium: Lavender colored.

Enzymatic zone: Fair, 7-8 mm. wide.

8. Potato plug.
Growth: At first (4 days) cream-colored, later pink patches appear and finally (15 days) all growth becomes of a dark red color.
Aerial mycelium: At first white, later (14 days) surface is changed to pink, then to red.
9. Carrot, 25°, 22 days.
Growth: At first (7 days). restricted, brownish; later the growth is spreading, thin, smooth, of a characteristic red-beet color.
Aerial mycelium: None.
Color of plug: Unchanged.
10. Gelatin, 18°, 35 days.
Growth: Yellowish red to dragon pink (Rdg. XII, 6') surface growth; growth also consisting of colorless flakes on bottom of liquefied portion.
Aerial mycelium: Cottony, hemosa pink (Rdg. I, 1) color.
Soluble pigment: Brown.
Liquefaction: Rapid (1½-2 cm. in 35 days).
11. Synthetic solution.
Growth: Small, white, cottony colonies all throughout medium.
Aerial mycelium: Faint pink.
Soluble pigment: None to faint brown.
 When glycerin is substituted for saccharose the colonies are deep red.
12. Milk, 37°. Soluble brown pigment.
Growth (25°): Abundant, red colored surface growth; rose-colored aerial mycelium.
Coagulation: 4-6 days.
Peptonization: Begins soon after coagulation is complete, advances very slowly, so that in 50 days not all the coagulum is digested.
Change of reaction: Unchanged.
13. Glucose broth, 25°, 12 days.
Growth: Thick, flaky mass on bottom of tube.
Aerial mycelium: None.
Soluble pigment: Brown to none.
14. Utilization of different carbon compounds.

Arabinose.....	0	Dextrose.....	4	Lactose.....	2
Glycerin.....	3	Saccharose.....	1	Maltose.....	4
Cellulose.....	4	Mannite.....	1	Starch.....	5
Organic acids.....	1-2				
(acetate)					

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Excellent with different sources of carbon.
 2. *Proteolytic action*: Fair in milk; fair on gelatin, both in presence and absence of starch; faint on glucose broth.
 3. *Change of reaction*: No change of slight alkalinity with NaNO_3 as source of nitrogen and different carbon compounds as sources of energy; faintly alkaline in acid gelatin; very distinctly acid in glucose broth.
 4. *Inversion of sugar*: Positive.
 5. *Diastatic action*: Scant in solution, fair on plate.
 6. *Growth on cellulose*: Very good on paper in solution.
- Hab.* New Jersey orchard and California upland soils.

Actinomyces roseus Krainsky 1914, p. 682, emend. Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Numerous open and closed spirals produced on the different synthetic media; type of spirals dextrorose.

2. Conidia.

Synthetic agar: Oval, 1.0 to 1.2 x 1.5 to 3.0 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Thin, spreading, colorless, penetrating deep into the medium.

Aerial mycelium: Thin, pale brownish vinaceous (Rdg. XXXIX, 5'''-f); property may be lost on successive transfers, but can easily be regained on transferring to favorable media.

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Spreading, colorless, growing deep into the medium.

Aerial mycelium: White to rose colored.

Soluble pigment: None.

3. Glucose agar.

Growth: Extensive, colorless, spreading on and below the surface of the medium, edge entire.

Aerial mycelium: Covering all surface, except wide margin; flesh-pink color (Rdg. XIII, 5'-f), with white margin.

Soluble pigment: None.

4. Nutrient agar.

Growth: White, later turning yellowish.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood serum, 37°.

Growth: Thin gray smear, later (15 days) turning brown.

Aerial mycelium: None.

Soluble pigment: None, often dark.

Liquefaction: None.

6. Egg-media, 37°.

Growth: Thin, spreading, greenish-yellow.

Aerial mycelium: None.

Soluble pigment: Narrow purple zone around growth.

7. Starch plate, 25°, 12 days.

Growth: Spreading, colorless.

Aerial mycelium: White cottony tufts all over surface, with shade of pink.

Enzymatic zone: 6-7 mm. wide.

8. Potato plug.

Growth: Much wrinkled, brownish.

Aerial mycelium: None.

Color of plug: Brown; on continued cultivation property lost, color of plug remaining unchanged.

9. Carrot.

Growth: Scant, restricted, wrinkled, light brownish.

Aerial mycelium: None.

Color of plug: Unchanged.

10. Gelatin, 18°.

Growth: Small, cream-colored colonies, sinking into the medium.

Aerial mycelium: None or thin white.

Soluble pigment: Brown, spreading into the unliquefied portion.

Liquefaction: Slow.

11. Synthetic solution.

Growth: Flakes throughout liquid.

Aerial mycelium: None.

Soluble pigment: None.

12. Milk, 37°.

Growth (25°): Brownish ring on surface in contact with glass.

Coagulation: None.

Hydrolysis: 10-15 days.

Change of reaction: Strongly alkaline (4).

13. Glucose broth.

Growth: Flakes on bottom, creamy ring on surface.

Aerial mycelium: None.

Soluble pigment: Brown.

14. Utilization of different carbon compounds.

Arabinose.....	1	Dextrose.....	3	Starch.....	3
Glycerin.....	3	Saccharose.....	2		
Cellulose.....	1	Mannite.....	1		
Organic acids.....	0-1				

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Good, in presence of different sources of carbon.
 2. *Proteolytic action:* Faint in milk.
 3. *Change of reaction:* Slight increase in alkalinity with NaNO_3 as source of nitrogen and different carbon compounds; strong alkalinity in milk.
 4. *Inversion of sugar:* None.
 5. *Diastatic action:* Very good, all starch used up in 14 days from a 1 per cent solution; on continued cultivation, culture loses somewhat its diastatic power, giving only a fair action on starch, by plate method.
 6. *Growth on cellulose:* None.
- Hab.* Isolated from New Jersey garden soil and identified by Waksman and Curtis (1916) as belonging to the above species although comparison with Krainsky's culture, as is the case of the other cultures, was impossible.

Actinomyces ruber Krainsky 1914, p. 686

I. MORPHOLOGY.

1. Spirals.

Usually none on all media studied. Mycelium consists of straight branching hyphae, often radiating from a common center. A few spirals may be formed.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Abundant, spreading, developing deep into the medium; red colored.

Aerial mycelium: Abundant, cottony, chrom-orange color (Rdg. II, 11).

Soluble pigment: None.

2. Calcium malate-glycerin agar.
Growth: Spreading, orange colored (Rdg. III, 15) with lighter margin penetrating deep into the medium.
Aerial mycelium: Cottony, cadmium yellow (Rdg. III, 17), developing at an early date.
Soluble pigment: None.
3. Glucose agar.
Growth: Restricted, abundant, chiefly on surface, margin entire, coral red (Rdg. XIII, 5') color, with yellowish growth in the medium.
Aerial mycelium: Powdery, thin, white, with pink tinge, leaving uncovered margin.
Soluble pigment: None.
4. Nutrient agar.
Growth: Restricted, elevated, wrinkled surface growth, olive-green (Rdg. XXXI, 25'') color.
Aerial mycelium: Thin, ash-gray.
Soluble pigment: Brown.
5. Blood agar, 37°.
Growth: Green.
Aerial mycelium: None.
Soluble pigment: Dark-gray, slowly spreading.
Hemolysis: Narrow colorless zone.
6. Blood serum, 37°.
Growth: Yellow, with red center.
Aerial mycelium: None.
Soluble pigment: Brown, spreading.
Liquefaction: None.
7. Egg-media, 37°.
Growth: Spreading, radially wrinkled, brick-red color.
Aerial mycelium: None at first, later (15 days) rose-color on edge of growth.
Soluble pigment: Faint dark zone.
8. Starch plate, 15 days.
Growth: Abundant, spreading, red.
Aerial mycelium: Pinkish-red.
Enzymatic zone: Fair, 8-10 mm. wide, not perfectly clear.
9. Potato plug, 25°.
Growth: Elevated, wrinkled, greenish (4 days).
Aerial mycelium: Red, with yellow margin.
Color of plug: Black zone around growth.
10. Carrot, 25°, 22 days.
Growth: Abundant, spreading, raised, brownish colored.
Aerial mycelium: Cottony, light salmon orange (Rdg. II, 11-d), all over surface of growth.
Color of plug: Dark brown.
11. Gelatin, 18°, 30 days.
Growth: Yellow flakes.
Aerial mycelium: None.
Soluble pigment: Brown, spreading.
Liquefaction: Medium.
12. Synthetic solution.
Growth: Colorless flakes throughout the medium.
Aerial mycelium: None.
Soluble pigment: None.

13. Milk, 37°.

Growth (25°, 20 days): Dark surface ring, with tinge of red.

Coagulation: 3-4 days.

Peptonization: Begins in 3-4 days, advances very rapidly, and is completed in about 10 days.

Change of reaction: Fairly alkaline (2).

14. Glucose broth, 25°, 12 days.

Growth: Small spongy colonies on surface of liquid with a red ring in contact with glass.

Aerial mycelium: Powdery, white, in upper portion of ring.

Soluble pigment: None.

15. Utilization of different carbon compounds. When glycerin is substituted for saccharose, into the synthetic solution, a characteristic growth is produced consisting of colonies throughout the medium having a red center and a wide colorless margin; a pink aerial mycelium is formed on the surface colonies.

<i>Arabinose</i>	0-trace	<i>Dextrose</i>	3	<i>Lactose</i>	3
<i>Glycerin</i>	3	<i>Saccharose</i>	2-5	<i>Maltose</i>	4
<i>Cellulose</i>	2	<i>Mannite</i>	2-3	<i>Starch</i>	4
<i>Organic acids</i>	0 (oxalate)-2 (acetate).				

16. Utilization of different nitrogen compounds.

<i>Ammonium sulfate</i>	2	<i>Ammonium carbonate</i>	1
<i>Sodium nitrile</i>	1	<i>Acetamide</i>	1-2
<i>Sodium nitrate</i>	2	<i>Leucin</i>	3-4
<i>Glycocoll</i>	2-5	<i>Peptone</i>	3-4
<i>Asparagin</i>	3-5	<i>Casein</i>	2-3
<i>Egg-albumin</i>	2-4	<i>Fibrin</i>	3-5
<i>Urea</i>	3		

III. BIOCHEMICAL FEATURES.

1. *Nitrile formation*: Faint to fair with most sources of carbon, good with maltose and lactose and excellent with arabinose, although the growth on this source of carbon was only very limited.
 2. *Proteolytic action*: Good on milk, good to very good on peptone, casein, fibrin; faint on egg-albumin.
 3. *Change of reaction*: In most cases distinctly alkaline, with NaNO_3 as source of nitrogen, and with different sources of carbon; with dextrose, mannite and glycerin reaction is unchanged; reaction is unchanged with most nitrogen sources and glycerin as source of carbon, acid with glycocoll and leucin, alkaline with peptone; distinctly acid in alkaline glucose broth (pH changed from 7.9 to 5.6 in 15 days).
 4. *Inversion of sugar*: Positive.
 5. *Diastatic action*: Fair on plate: height of hydrolyzed starch by tube method, above control, 14-16 mm.
 6. *Growth on cellulose*: Good with all methods; no clear zone formed on plate.
- Hab.* Received from Dr. C. B. Lipman, who isolated it from the forming soil of Tortugas Island.

Actinomyces rutgersensis Waksman and Curtis

This organism corresponds very closely, on certain media and in some biochemical characters, with *A. diastaticus* described by Krainsky (22). This organism, as well as the *A. diastaticus* and *A. lipmanii*, represent closely related forms (culturally) and are among the most common of the soil actinomycetes.

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: Abundant, both close and open type; hyphae fine, long, branching.

2. Conidia.

Synthetic agar: Spherical and oval, 1.0 to 1.2 μ in diameter; tendency to bi-polar staining.

II. CULTURAL CHARACTERISTICS.

1. *Synthetic agar*:

Growth: Fair, thin, spreading, penetrating deep into the medium, at first colorless, later becoming brownish to almost black, particularly on repeated transfers.

Aerial mycelium: At first thin white, later becoming pale dull-gray (Rdg. LIII, C. G.).

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Spreading, developing deep into the medium, cream-colored; edge myceloid.

Aerial mycelium: Thin, covering all the growth, leaving narrow edge uncovered; pale dull-gray in color, with white patches.

Soluble pigment: None.

3. Glucose agar.

Growth: Abundant, brown colored on surface, penetrating only very slightly into the medium, edge myceloid; in 30 days color of growth is black, with wide cream-colored margin.

Aerial mycelium: None in 15 days, white patches in 30 days.

4. Nutrient agar.

Growth: Thin, wrinkled, cream-colored.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood serum, 37°.

Growth: Restricted, glossy, gray smear.

Aerial mycelium: None.

Soluble pigment: None.

Liquefaction: None.

6. Egg-media, 37°.

Growth: Thin, gray colored smear.

Aerial mycelium: Thin, powdery, white.

Soluble pigment: Purple spreading pigment appears late (30 days).

7. Starch plate, 25°, 12 days.

Growth: Gray, spreading.

Aerial mycelium: Gray.

Enzymatic zone: Fair to good, 8-10 mm. wide.

8. Potato plug.
Growth: Abundant, much folded, white-gray colored at first (4 days), later becoming brownish, till finally (30 days) it is all brown.
Aerial mycelium: White patches appearing late (15 days).
Color of plug: Unchanged.
9. Carrot, 25°, 22 days.
Growth: Large, round, restricted, cream-colored colonies; surface smooth, dry, much raised.
Aerial mycelium: None.
Color of plug: Unchanged.
10. Gelatin, 18°, 30 days.
Growth: Cream-colored spreading with flakes dropping to bottom of tube.
Aerial mycelium: White, thin patches over growth.
Soluble pigment: None at first; on continued cultivation, a light brown color is produced in liquefied portion only.
Liquefaction: Medium; all tube liquefied in 30-35 days; in the presence of 1 per cent starch only half of the tube is liquefied in the same period of time.
11. Synthetic solution (no growth with saccharose; glycerin used in this solution).
Growth: Thin white pellicle; few flakes through medium.
Aerial mycelium: Thin, white.
Soluble pigment: None.
12. Milk, 37°.
Growth (25°): Cream-colored ring on surface.
Coagulation: 4-6 days.
Peptonization: Begins as soon as coagulation is complete, advances very slowly, so that in 50 days not all the coagulum is digested.
Change of reaction: Fairly alkaline (2).
13. Glucose broth, 25°, 12 days.
Growth: Small colorless colonies on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Good to very good with different sources of carbon.
2. *Proteolytic action*: Good in milk; very good on gelatin, both in presence and absence of starch.
3. *Change of reaction*: Fair alkalinity in milk; practically unchanged in glucose broth; distinctly alkaline in gelatin, both in presence and absence of starch.
4. *Inversion of sugar*: None.
5. *Diastatic action*: Excellent; all starch used up in 7 days in a 1 per cent solution; fair to good on plate.
6. *Growth on cellulose*: Scant.

Hab. Common soil organism. Isolated from New Jersey garden orchard and timothy soils; Louisiana, California, North Dakota, Alaska, Texas, and Colorado soils.

Actinomyces scabies (Thaxter) Güssow (Syn. *Streptothrix chromogenus* Gasperini, *Oospora scabies* Thaxter)

I. MORPHOLOGY.

1. Spirals:

This organism did not form many spirals on the media under the conditions as studied; the aerial myphae are usually long and branched; often few spirals are produced.

Synthetic agar: Wavy or slightly curved; on other media (cellulose) agar spiral formation took place; the spirals are of a dextrorose type.

2. Conidia.

Conidia are produced readily on most synthetic media; these are more or less cylindrical, $0.8-1.0 \times 1.2-1.5 \mu$.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Abundant, cream-colored, chiefly on surface of medium, wrinkled, raised.

Aerial mycelium: White, scarce; some strains may not produce any at all.

Soluble pigment: None at first, later a brown pigment may develop.

2. Calcium malate-glycerin agar.

Growth: Good, spreading both on surface and into medium; faint yellowish in color, later turning dark.

Aerial mycelium: Thin layer all over colony, only leaving edge uncovered, mouse-gray with white margin and patches.

Soluble pigment: None.

3. Glucose agar.

Growth: Restricted, folded, cream-colored, edge entire.

Aerial mycelium: None.

Soluble pigment: None; surface agar around medium colored milky white.

4. Nutrient agar.

Growth: Growth consists of round, entire colonies; surface at first smooth, later raised, lichnoid, often becoming wrinkled; color white to straw colored, opalescent to opaque.

Aerial mycelium: Usually absent, often a scant white is produced.

Soluble pigment: Brown, spreading.

5. Blood agar, 37° , 15 days.

Growth: Brownish to gray.

Aerial mycelium: None.

Soluble pigment: Dark brown.

Hemolysis: None.

6. Blood serum, 37° , 7 days.

Growth: Slow, restricted, much folded, yellowish to brown colonies.

Aerial mycelium: None.

Soluble pigment: Brown, forming a zone around growth.

Liquefaction: None.

7. Egg-media, 37° , 7 days.

Growth: Small, wrinkled, brown colonies, later becoming dark brown.

Aerial mycelium: None.

Soluble pigment: Narrow purplish to black zone.

8. Starch plate, 12 days.

Growth: Thin, transparent, spreading.

Aerial mycelium: Scant white.

Enzymatic zone: Questionable diastase production.

9. Potato plug, 7 days.

Growth: Gray, opalescent, later turning jet black, wrinkled colonies covering all the plug.

Aerial mycelium: None; in some strains an ash gray mycelium appears.

Color of plug: Black.

10. Carrot.

Growth: Scant, thin, yellowish, semitransparent.

Aerial mycelium: None.

Color of plug: Unchanged.

11. Gelatin, 18°.

Growth: Cream-colored, turning brown in portions exposed to the air.

Aerial mycelium: Scant white in 37 days.

Soluble pigment: Deep brown.

Liquefaction: Slow at first, later becoming more rapid so that in 35 days 2.7 cm. of the gelatin is digested.

12. Synthetic solution (glycerin in place of saccharose).

Growth: An abundant mass of colorless flakes and colonies on bottom of tube.

Aerial mycelium: None.

Soluble pigment: None.

13. Milk, 37°.

Deep brown pigment begins to develop in 7 days in the form of a surface ring, and in 30 days the whole tube turns brown.

Growth: Brown colored ring with greenish tinge at 25°.

Coagulation: 5-10 days; certain strains many not show any coagulation at all, but a slow hydrolysis.

Peptonization: Begins soon after coagulation is complete (5-10 days), proceeds at fair speed, and is completed in 15-30 days.

Change of reaction: Fairly alkaline (2).

14. Glucose broth.

Growth: Growth on the surface in the form of a ring, consisting of many small colonies, which soon fuse together; this may settle to the bottom.

Aerial mycelium: None.

Soluble pigment: Brown, dissolving downward.

15. Utilization of different carbon compounds.

Arabinose.....	4	Dextrose.....	3	Lactose.....	4
Glycerin.....	3	Saccharose.....	2-3	Starch.....	3
Cellulose.....	1-2	Mannite.....	3		
Organic acids.....	1				

16. Utilization of different nitrogen compounds.

Ammonium sulfate.....	0 (T)	Ammonium carbonate.....	0 (T)
Sodium nitrite.....	1-3	Acetamide.....	1-2
Sodium nitrate.....	1-2	Leucin.....	2-3
Glycocoll.....	2-3	Peptone.....	3-4
		(reaction alkaline, ammonia produced late, after 20 days).	
Asparagin.....	2	Fibrin.....	3-5
		(distinct ammonia accumulation).	
Egg-albumin.....	2-3	Casein.....	3-4
Urea.....	1-2		

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* None to mere traces with nearly all sources of carbon, with the exception of glycerin when a fair nitrite formation took place; often none is found even with glycerin.

2. *Proteolytic action*: Fair on milk, weak on peptone, casein, fibrin, and egg-albumin, although a sufficient amount of the material is split to produce a very good growth; fair on gelatin.
3. *Change of reaction*: With NaNO_3 as a source of nitrogen, the medium becomes alkaline with nearly all sources of carbon studied; distinctly alkaline in acid gelatin (pH 6.2 changed to 7.9 in 35 days at 18°); alkaline with peptone, casein, fibrin, glyocoll, etc., faint acidity with leucin and acetamide (glycerin as source of energy); distinctly alkaline in milk; unchanged in alkaline glucose broth.
4. *Inversion of sugar*: Positive.
5. *Diastatic action*: Questionable on plate, hydrolysis only to erythro-reaction; same on tube.
6. *Production of tyrosinase*: Very good. Although most organisms made a good growth on tyrosin agar (with tyrosin as the only source of nitrogen), only some strains of *A. scabies*, and *A. chromogenus* 205 produced a soluble dark brown pigment, spreading readily through the medium. This would tend to indicate that these two organisms are the only ones that are able to produce a true tyrosinase, or, if, as Beijerinck (3) suggested, tyrosinase is a mixture of two enzymes, only these two organisms of the whole group are able to produce both enzymes.
7. *Growth on cellulose*: Fair with certain methods (plate and reprecipitated cellulose in solution); none on paper in solution; clear zone is not formed on plate.

Hab. Isolated from potato scab, also from the soil.

A number of cultures received from other investigators were compared with the strain described above, and many of them showed distinctive differences, particularly as to the production of a brown soluble pigment on organic media and aerial mycelium on synthetic media. Some strains attacked the carrot and potato plug very readily, so that within 2 weeks the plug was all shrivelled up and covered with ash-gray powdery aerial mycelium; one strain made no growth on carrot and only a scant smear was produced on potato; several other strains fell between the two extremes. The weakly growing strains which made a scant or poor growth on potato and carrot, without pigmenting the plug dark brown, produced also no dark pigment on tyrosin agar and attacked the milk proteins only slowly.

Actinomyces verne Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: No spirals; the branching of the hyphae is so close as to produce the impression of making whirls.

Starch agar: Few short coiled side branches.

2. Conidia.

Synthetic agar: None demonstrated.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Abundant, spreading, wrinkled, much elevated above surface, also developing into medium, margin lichnoid, surface glossy; color yellowish at first, later developing a brown tinge.

Aerial mycelium: No true aerial mycelium demonstrated.

Soluble pigment: Elm-green (Rdg. XVII, 27-Km) diffusing through medium; on repeated transfer, the culture produces, instead of a green pigment, a deep brown soluble pigment.

2. Calcium malate-glycerin agar.
Growth: Thin, restricted, wrinkled, chiefly on surface, color avellaneous (Rdg. XL, 17'''-b).
Aerial mycelium: None in 20 days, later scant white.
Soluble pigment: Faint brown.
3. Glucose agar.
Growth: Abundant, much folded, chiefly on surface of medium; center raised, edge entire; color gray with purplish-brown tinge.
Aerial mycelium: None.
Soluble pigment: Faint, brown.
4. Nutrient agar.
Growth: Small grayish colonies, with depression in center, later becoming wrinkled.
Aerial mycelium: None.
Soluble pigment: None.
5. Blood serum, 37°.
Growth: Restricted, cream-colored.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: Medium (10-12 days).
6. Egg-media, 37°.
Growth: Thin, much wrinkled, cream-colored.
Aerial mycelium: None.
Soluble pigment: None.
7. Starch plate, 25°, 16 days.
Growth: Brownish colored, scant, restricted growth.
Aerial mycelium: None to a faint brown pellicle on surface of growth.
Enzymatic zone: 8-10 mm. wide.
8. Potato plug.
Growth: Wrinkled, cream-colored (4 days), later (15 days) becoming gray.
Aerial mycelium: None, sometimes scanty white mycelium is produced.
Color of plug: Becomes faint brown with age of culture (30 days).
9. Carrot, 25°, 22 days.
Growth: Spreading, raised, much folded, brownish.
Aerial mycelium: None.
Color of plug: Unchanged.
10. Gelatin, 18°, 30 days.
Growth: Small cream-colored colonies.
Aerial mycelium: None.
Soluble pigment: Green, property lost on continued cultivation.
Liquefaction: Rapid to medium; liquefied portion in tube 1.5 cm. deep in 30 days.
11. Synthetic solution.
Growth: Small colonies on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.
When glycerin is substituted for saccharose, there is formed a mass of brownish flakes on bottom of tube, with a faint brown soluble pigment.
12. Milk, 37°.
Growth (25°): Pinkish-brown ring on surface.
Coagulation: 4-5 days.
Peptonization: Begins in 5 days, advances rapidly and completed in 18-25 days.
Change of reaction: Fairly alkaline (2).

13. Glucose broth.

Growth: Small flakes on bottom of tube.*Aerial mycelium:* None.*Soluble pigment:* Faint brown to none.

14. Utilization of different carbon compounds.

<i>Arabinose</i>	0	<i>Dextrose</i>	1	<i>Lactose</i>	2
<i>Glycerin</i>	1	<i>Saccharose</i>	1-2	<i>Maltose</i>	2
<i>Cellulose</i>	1-3	<i>Mannite</i>	3	<i>Starch</i>	1

15. Utilization of different nitrogen compounds (glycerin as a source of energy).

<i>Ammonium sulfate</i>	0	<i>Ammonium carbonate</i>	0
<i>Sodium nitrite</i>	1	<i>Acetamide</i>	1
<i>Sodium nitrate</i>	1	<i>Leucin</i>	3
<i>Glycocol</i>	3	<i>Casein</i>	2-3
<i>Asparagin</i>	2-3	<i>Fibrin</i>	2
<i>Egg-albumin</i>	2	<i>Urea</i>	1
<i>Peptone</i>	3		

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Good with different sources of carbon.
2. *Proteolytic action:* Very good in milk and on the different pure proteins (fibrin, casein, egg-albumin).
3. *Change of reaction:* No change, slight acidity or slight alkalinity with NaNO_3 , as source of nitrogen, depending on the source of carbon; unchanged or faint alkalinity with different proteins and amino acids, except with leucin, where the change of reaction is towards acidity.
4. *Inversion of sugar:* Positive.
5. *Diastatic action:* Very good, starch reduced chiefly to erythrodextrin; all starch disappeared in 14 days in a 1 per cent solution. Diastatic power somewhat deteriorated on continued cultivation, giving only a good reaction (3).
6. *Growth on cellulose:* Usually good, when cellulose, either in the form of paper or dissolved and reprecipitated, is the only source of carbon, but none on plate.

Hab. Isolated from California upland soil.*Actinomyces violaceus-caesari* Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: Numerous open spirals.*Calcium-malate agar:* Broad spirals; both spirals and straight aerial mycelium break up into spores.*Dextrose agar:* Numerous, open; some have corkscrew effect; spirals dextro-rose.

2. Conidia.

Calcium-malate agar: Oval-shaped to elongated.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 15 days.

Growth: Colonies glossy, much wrinkled, gray at first, later becoming bluish.*Aerial mycelium:* Appears late, white with no shade of gray.*Soluble pigment:* Plum-purple (Rdg. XXIV, 57-m), turning darker to almost brown with age of culture.

2. Calcium malate-glycerin agar, 15 days.
Growth: Restricted, developing deep into medium, only aerial mycelium on surface of medium; color of growth is blue.
Aerial mycelium: White, with purplish tinge, due to pigment of underlying growth.
Soluble pigment: Faint blue, rapidly spreading.
3. Glucose agar, 15 days.
Growth: Restricted, on surface, penetrating to some extent into the medium; at first gray, later turning red to almost brick-red.
Aerial mycelium: White patches over surface of growth.
Soluble pigment: None.
4. Nutrient agar, 15 days.
Growth: Thin, cream-colored smear.
Aerial mycelium: None.
Soluble pigment: None.
5. Blood serum, 37°.
Growth: Small, restricted, gray colonies, developing only in 15 days.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: None.
6. Egg-media, 37°.
Growth: Thin, finely wrinkled, of a grayish color (5-6 days), later (15 days) showing a dark center.
Aerial mycelium: None.
Soluble pigment: None.
7. Starch plate, 12 days.
Growth: Individual, round, restricted colonies, of a bluish violet color.
Aerial mycelium: In the form of tufts, gray in centre and white at the periphery.
Soluble pigment: None.
Enzymatic zone: 12-14 mm. wide, but hydrolysis is not complete (reddish tinge on addition of iodine solution); the starch is hydrolyzed chiefly to the erythro-dextrin stage.
8. Potato plug.
Growth: Cream-colored, wrinkled, in 4-5 days; in 15 days color of growth turns yellowish.
Aerial mycelium: None.
Color of plug: Unchanged.
9. Carrot: No growth.
10. Gelatin, 18°, 35 days.
Growth: Small, cream-colored colonies on surface, may drop to bottom of liquefied portion.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: Medium or slow.
11. Synthetic solution, 15 days.
Growth: Small flakes on bottom of tube.
Aerial mycelium: None.
Soluble pigment: Blue, property almost lost with age of organism on continued cultivation. On replacing the saccharose by glycerin, the blue color is formed readily.

12. Milk, 37°.

Growth: Gray ring on surface.

Coagulation: 10-12 days; sometimes milk is not clotted.

Peptonization: Slow, complete only in 50 days.

Change of reaction: Faintly alkaline (1).

13. Glucose broth, 12 days.

Growth: Fine, colorless, flaky growth on bottom of flask or tube.

Aerial mycelium: None.

Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair, with starch as a source of carbon; none, with saccharose and glycerin.

2. *Proteolytic action:* Fair in milk and gelatin.

3. *Change of reaction:* With NaNO_3 as a source of nitrogen, the medium turns alkaline for all sources of carbon used; faintly alkaline on milk.

4. *Inversion of sugar:* None.

5. *Diastatic action:* Very good amylolytic power, but weak saccharogenic power; 1 per cent starch all reduced to the erythro-reaction in 14 days; very good by tube and plate methods, but hydrolysis of the starch is in all cases incomplete, reduced chiefly to the erythro-reaction.

6. *Growth on cellulose:* Faint to fair, particularly on paper in synthetic solution; no ring formation on plate.

Hab. Isolated from California upland soil.

A. violaceus-niger described originally (45) and several strains of the chromogenus group were found on further cultivation to be closely related to this species and are therefore omitted here. The *A. violaceus-caesari*, although a distinct species resembles in certain respects the chromogenus forms.

Actinomyces violaceus-ruber Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: Numerous, of the open type.

Dextrose agar: Short, imperfect spirals; both spirals and straight mycelium breaking up into spores; spirals dextrorose.

2. Conidia.

Synthetic agar: Oval and rod-shaped, 0.8 to 1.5×0.7 to 1.0μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 15 days.

Growth: Thin, spreading, growing deep into medium, uncolored at first, soon changing to red and then blue.

Aerial mycelium: Thin, powdery, covering all colony, at first white, later turning mouse-gray (Rdg. LI, 15''').

Soluble pigment: A red soluble pigment is first produced which changes to cyanine blue (Rdg. IX, 51-m). The production of the pigment and successive changes can be readily explained by the change in reaction of the medium. The organism produces a soluble pigment, which acts as an indicator; it changes from red to blue at pH 7.4-7.6. The synthetic agar has a reaction of pH 7.0, therefore the pigment when produced is red. The medium becomes more alkaline as a result of the growth of the organism; when the reaction changes

to P_H 7.4-7.6, the red pigment changes to blue. The rapidity of the change of pigment differs with the strains of the organism, depending probably on the rapidity of alkali production. When studied in solution, the change of color is not very sharp, but somewhat gradual, varying from red to red brown and blue. This pigment is only one of two or even three pigments present in the mixture; it may therefore often be obscured.

2. Calcium malate-glycerin agar, 15 days.

Growth: Thin, spreading, penetrating to some extent into the medium; rose red in color.

Aerial mycelium: Thin, powdery, ash-gray, with white edge; abundant, all over growth.

Soluble pigment: None in 15 days; red pigment slowly spreading appears in 35 days, the lack of blue pigment is no doubt due to the fact that this medium does not turn alkaline to the indicator produced by the organism.

3. Glucose agar, 15 days.

Growth: Spreading, more on surface than into medium, edge regular; of a brick-red color, later turning almost black.

Aerial mycelium: Thin, cottony, ash-gray, with white edge; abundant all over growth.

Soluble pigment: None at first: faint red to reddish brown pigment appears in 35 days.

4. Nutrient agar.

Growth: At first (4 days) white, later becoming red, with white margin.

Aerial mycelium: White, appearing only in 10-12 days.

Soluble pigment: Blue, spreading very slow.

5. Blood agar, 37°.

Growth: A slightly elevated, crumpled, spreading, red growth appears early (2-3 days), surface glistening

Aerial mycelium: None.

Soluble pigment: Faint brown, limited to reverse of growth only.

Hemolysis: Strong.

6. Blood serum, 37°.

Growth: Thin, brownish spreading smear in 3-4 days, later turning red; surface glistening.

Aerial mycelium: None.

Soluble pigment: Soluble red pigment (7 days).

Liquefaction: None.

7. Egg-media, 37° (also at 25°, although growth is somewhat slower).

Growth: Thin, spreading brown smear in 5-6 days.

Aerial mycelium: White, all over growth, with a bluish tinge, developing in 2 weeks.

Soluble pigment: Blue, often none.

8. Starch plate, 12 days.

Growth: Spreading, pink-colored.

Aerial mycelium: Powdery, ash-gray color, all over surface of growth; the marginal zone is often white.

Soluble pigment: None.

Enzymatic zone: 6-8 mm. wide.

9. Potato plug.

Growth: Small brownish colonies appearing in 3-4 days; in about 2 weeks growth is found to be abundant, folded, of a grayish color.

Aerial mycelium: Abundant, white, with ash-gray patches in 7-8 days; bluish tinge in 2 weeks.

Color of plug: Blue in 4-5 days.

10. Carrot.

Growth: Abundant, spreading, much folded, lichnoid, cream-colored at first, later turning brownish.

Aerial mycelium: Gray, powdery all over surface, with shade of pink developing in 15 days.

Color of plug: Bright pink around growth, with dark brown pigment spreading over the plug.

11. Gelatin, 18°, 18 days.

Growth: Spreading, dense, cream-colored at first, the underlying part changing to pink or blue depending on reaction of gelatin to start with; quite often the color remains grayish.

Aerial mycelium: White or none at all, when the flaky growth drops to the bottom of the liquefied portion.

Soluble pigment: None, sometimes blue.

Liquefaction: Slow to medium; in presence of starch it is more rapid; 1½ cm. of depth of tube liquefied in 30 days.

12. Synthetic solution, 15 days.

Growth: Numerous, small, round colonies throughout the medium.

Aerial mycelium: Scant, gray.

Soluble pigment: Red changing to blue.

13. Milk, 37°, 15 days.

Growth: Gray with shade of red or blue pigmented ring on surface of milk in contact with glass.

Coagulation: Usually none; often it takes place in 9–10 days, followed by a very slow peptonization; the clot when produced is soft.

Hydrolysis: 12–15 days, complete; hydrolyzed portion has a pinkish tinge.

Change of reaction: Distinctly alkaline (5).

14. Glucose broth, 15 days.

Growth: Solid grayish ring on surface, close to tube, none throughout medium.

Aerial mycelium: White.

Soluble pigment: Blue.

15. Utilization of different carbon compounds.

<i>Arabinose</i>	1	<i>Dextrose</i>	5	<i>Lactose</i>	5
<i>Glycerin</i>	3	<i>Saccharose</i>	3	<i>Starch</i>	5
<i>Cellulose</i>	2–3	<i>Organic acids</i>	0–1	<i>Maltose</i>	3–4
<i>Mannite</i>	4				

16. Utilization of different nitrogen compounds.

<i>Ammonium sulfate</i>	0	<i>Ammonium carbonate</i>	0
<i>Sodium nitrite</i>	3	<i>Acetamide</i>	2
<i>Sodium nitrate</i>	1	<i>Leucin</i>	3
<i>Glycocoll</i>	3–4	<i>Casein</i>	3–4
<i>Asparagin</i>	3–4	<i>Fibrin</i>	3
<i>Egg-albumin</i>	2	<i>Urea</i>	1–2
<i>Peptone</i>	3–4		

III. BIOCHEMICAL FEATURES

1. *Nitrite formation:* Excellent with nearly all sources of carbon.
2. *Proteolytic action:* Good on milk and on gelatin.
3. *Change of reaction:* With NaNO_3 as source of nitrogen, the medium is made alkaline with the following sources of carbon: salts of organic acids, maltose, dextrose, lactose, etc. When grown on acid gelatin, the reaction changes to

alkaline (from P_H 6.2 to P_H 8.0) both in presence and absence of starch; distinctly alkaline in milk; alkaline glucose broth made acid (P_H changed from 7.9 to 6.6).

6. *Inversion of sugar*: Usually positive.
5. *Diastatic action*: Good; 1 per cent starch not used up entirely in 14 days; good by tube and plate methods.
6. *Growth on cellulose*: Fair to good, depending on method used; cellulose used fairly well; clear zone formed on cellulose plate.

Hab. Isolated from Iowa and California adobe soils.

Actinomyces viridochromogenus Krainsky 1914, p. 684, emend.
Waksman and Curtis

Isolated numerous times from different soils; strains differ chiefly in vigor of growth on artificial media; the following description is based chiefly on one strain (101).

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: Abundant, regular, 3-5 μ , in diameter.

Starch: Numerous, perfect, of an open type, 3-6 μ in diameter, occurring as side branches on long mycelia and terminal.

2. Conidia.

Synthetic agar: Short ovals to spheres, 1.25 x 1.25-1.5 μ .

Starch: Spherical to oval 1-1.25 x 1.0-1.5 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Spreading, cream-colored with dark center at first, later all surface growth becomes dark green; reverse yellowish to light cadmium (Rdg. IV, 19) ..

Aerial mycelium: Abundant, covering center first, then spreading all over surface; white, changing to light geladine green (Rdg. XLVII, 35'''-d).

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Thin, spreading, developing deep into medium; light gray at first, later changing to dark-green and almost black.

Aerial mycelium: Thin, powdery, all over surface, white at first, later becoming pale dull glaucous-blue (Rdg. XLII, 41'''-f).

Soluble pigment: None.

3. Glucose agar.

Growth: Spreading, abundant, developing chiefly on surface, partly into the medium, wrinkled, gray at first, later becoming black.

Aerial mycelium: White at first, later becoming greenish-blue.

Soluble pigment: Faint brown, with greenish tinge.

4. Nutrient agar, 15 days.

Growth: Abundant, restricted, gray growth, with greenish tinge.

Aerial mycelium: Powdery, white, with shade of green all over surface of growth.

Soluble pigment: Dark brown, spreading.

5. Blood agar, 37°, 15 days.

Growth: Dark gray, crumpled colonies, later turning brown.

Aerial mycelium: Scant white.

Soluble pigment: Dark, spreading.

Hemolysis: None.

6. Blood serum, 37°.

Growth: Small, glossy, brownish colonies in 4-5 days.
Aerial mycelium: White, with dark center.
Soluble pigment: Spreading, dark zone around growth.
Liquefaction: None.
7. Egg-media, 37°.

Growth: Extensive, spreading, brown growth in 5-6 days.
Aerial mycelium: White, with greenish patches in 5-6 days; pinkish tinge, when culture becomes older (15 days).
Soluble pigment: Dark all through medium.
8. Starch plate, 12 days.

Growth: Round, spreading, yellowish.
Aerial mycelium: Thin, greenish to gray, with zone formation.
Soluble pigment: None.
Enzymatic zone: Scant (2 mm. in 11 days).
9. Potato plug.

Growth: Abundant, gray-brown growth appears early (24 hours).
Aerial mycelium: Abundant, white, all over growth (3-4 days), developing a greenish tinge (15 days), which changes to pinkish with age of culture (30 days).
Color of plug: Black, spreading.
10. Carrot.

Growth: Restricted at first, later spreading, folded, cream-colored with dark shade developing from center of growth.
Aerial mycelium: Cottony, abundant, white, with shade of green.
Color of plug: Dark brown.
11. Gelatin, 18°, 30 days.

Growth: Colonies cream-colored, becoming greenish.
Aerial mycelium: Green, with yellow tinge.
Soluble pigment: Brown.
Liquefaction: Slow.
12. Synthetic solution.

Growth: Small flakes on side of tube; floating colonies; surface of a characteristic green color.
Aerial mycelium: Green.
Soluble pigment: None to shade of brown.
13. Milk, 37°. Soluble brown pigment.

Growth: Dark brown surface growth with shade of green in the form of a ring at 25°; none at 37°.
Coagulation: 5-6 days.
Peptonisation: Begins in 6 days, proceeds rapidly and in 12 days coagulum is all (imperfectly) digested; digestion much slower at 25°.
Hydrolysis: May take place instead of coagulation, particularly at 25°.
Change of reaction: Faintly alkaline (1).
14. Glucose broth, 12 days.

Growth: Dense solid ring on surface, in contact with glass, also small masses on surface of liquid; brownish, turning dark green in color.
Aerial mycelium: Thin, powdery, white to faint greenish.
Soluble pigment: Faint greenish to deep brown spreading downward.
15. Utilization of different carbon compounds.

Arabinose.....	3	Dextrose.....	4	Lactose.....	3
Glycerin.....	2	Saccharose.....	2	Maltose.....	5
Cellulose.....	1-3	Mannite.....	3	Starch.....	3
Organic acids.....	1-2 (lactate, malate, and tartrate)				

16. Utilization of different nitrogen compounds.

<i>Ammonium sulfate</i>	0	<i>Ammonium carbonate</i>	0
<i>Sodium nitrite</i>	1-3	<i>Acetamide</i>	1
<i>Sodium nitrate</i>	2	<i>Leucin</i>	3
<i>Glycocoll</i>	3-5	<i>Casein</i>	4-5
<i>Asparagin</i>	3	<i>Fibrin</i>	3
<i>Egg-albumin</i>	3	<i>Urea</i>	3
<i>Peptone</i>	4		

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Scant, with sucrose and glycerin as sources of energy, much better with starch.
2. *Proteolytic action*: This organism has a rather weak proteolytic action; fair on milk and gelatin; faint to fair on the proteins (fibrin, egg-albumin, casein).
3. *Change of reaction*: Alkaline with NaNO_3 as a source of nitrogen and with different carbon compounds as sources of carbon; faintly alkaline in milk; faint acidity with different proteins and amino acids as sources of nitrogen and glycerin as a source of carbon; faint acidity in glucose broth.
4. *Inversion of sugar*: None.
5. *Diastatic action*: Fair action in a 1 per cent starch solution; scant action on plate, zone 2 mm. in 11 days.
6. *Action on cellulose*: Usually good, but there was no zone formation on plate.

Hab. Isolated from New Jersey garden, California, Oregon adobe, Porto Rico and Texas soils.

Actinomyces 104

I. MORPHOLOGY.

1. Spirals.

Numerous, of the open type, on synthetic agar; few spirals observed on dextrose agar and glycerin.

II. CULTURAL CHARACTERISTICS. This organism is characterized by a very scant growth on the synthetic agar.

1. Synthetic agar.

Growth: Colorless, spreading, chiefly deep into the medium.

Aerial mycelium: Very thin, white; turning later grayish.

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Medium, spreading chiefly on surface; edge myceloid; cream-colored with brownish shade.

Aerial mycelium: None.

Soluble pigment: None.

3. Glucose agar.

Growth: Abundant, spreading growth, chiefly on surface, also penetrating below surface; edge myceloid, color of surface growth brown with wide colorless margin.

Aerial mycelium: Thin, powdery, all over surface, with narrow margin, white-colored.

Soluble pigment: Brown.

4. Nutrient agar.

Growth: Cream-colored, wrinkled, only on surface.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood serum, 37°.

Growth: Glossy, wrinkled, gray growth.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: Starts in 15 days.
6. Egg-media.

Growth: Thin, spreading, fine net-work, cream-colored with green tinge.
Aerial mycelium: None.
Soluble pigment: None.
7. Starch plate, 25°, 16 days.

Growth: Thin, spreading, cream-colored.
Aerial mycelium: None or a few grayish thin patches.
Enzymatic zone: 5-6 mm. wide; hydrolysis of the starch is incomplete.
8. Potato plug.

Growth: Abundant, much wrinkled, greenish at first (4 days), later becoming black with yellowish margin.
Aerial mycelium: None.
Color of plug: Faint black zone around growth appears late (15 days).
9. Carrot, 25°, 22 days.

Growth: Abundant, spreading, much folded, brownish.
Aerial mycelium: Scant, white patches.
Color of plug: Brown shade appears late (22 days).
10. Gelatin, 18°, 35 days.

Growth: Cream-colored flakes on bottom of liquefied portion.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: Rapid (3 cm. of depth of gelatin liquefied in tube in 35 days).
11. Synthetic solution.

Colonies: Scant flaky growth on bottom of tube.
Aerial mycelium: None.
Chromogenesis: None.
12. Milk, 37°.

Growth (25°): Pinkish surface ring in contact with glass.
Coagulation: 8-12 days.
Peptonization: Begins in 10-12 days and is nearly all completed 20-25 days.
Change of reaction: Fairly (2) to distinctly alkaline (3).
13. Glucose broth 25°, 12 days.

Growth: Thin, cream-colored pellicle on surface, some flakes on bottom.
Aerial mycelium: None.
Soluble pigment: None.
14. Utilization of different carbon compounds (NaNO₃ as source of nitrogen).

Arabinose.....	0	Dextrose.....	1	Lactose.....	1
Glycerin.....	1	Saccharose.....	1	Maltose.....	2
Cellulose.....	1	Mannite.....	1	Starch.....	1
Organic acids.....	0-1				

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Scant, though positive, with most sources of carbon.
2. *Proteolytic action:* Good on gelatin, both in presence and absence of starch; fair on milk and glucose broth.
3. *Change of reaction:* No change, slight acidity on alkalinity with NaNO₃ as source of nitrogen and different sources of carbon; faint alkalinity in gelatin and glucose broth and in milk.

4. *Inversion of sugar*: None.
5. *Diastatic action*: Fair in plate; good by tube method.
6. *Growth on cellulose*: None or very scant.
- Hab.* New Jersey orchard soil, 8 inches deep.

Actinomyces 145

I. MORPHOLOGY.

1. *Spirals*.
None on the media studied; hyphae usually coarse, branching, with a tendency to curl.
2. *Conidia*.
Oval-shaped to elliptical spores.

II. CULTURAL CHARACTERISTICS.

1. *Synthetic agar*.
Growth: Thin, colorless, spreading, developing deep into the medium.
Aerial mycelium: Thin, gray.
Soluble pigment: None.
2. *Calcium malate-glycerin agar*.
Growth: Scanty, colorless, raised in center, uneven edge.
Aerial mycelium: None.
Soluble pigment: None.
3. *Glucose agar*.
Growth: Good, spreading growth, cream-colored chiefly on surface, penetrating to a slight extent into the medium, center raised, edge myceloid.
Aerial mycelium: Abundant all over surface, pale mouse-gray (Rdg. LI, 15^{mm}-d) with white patches.
Soluble pigment: None.
4. *Nutrient agar*.
Growth: Thin, cream-colored.
Aerial mycelium: Gray.
Soluble pigment: None.
5. *Blood serum, 25°, 20 days*.
Growth: Faint cream-colored spots.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: None.
6. *Egg-media, 25°, 20 days*.
Growth: Abundant, spreading, dark brown, appearing late.
Aerial mycelium: None.
Soluble pigment: Narrow purplish zone around growth.
7. *Potato plug, 25°, 15 days*.
Growth: Small restricted colonies, gray at first (4 days), later (15 days) becoming dark.
Aerial mycelium: None at first, traces of white appearing in 15 days.
Color of plug: Black.
8. *Carrot, 25°, 22 days*.
Growth: Scant, spreading, raised in center, cream-colored.
Aerial mycelium: None.
Color of plug: Brown zone only in 22 days.

9. Starch plate, 25°, 15 days.
Growth: Rapidly spreading, cream-colored, with yellow reverse.
Aerial mycelium: Light buff-gray.
Enzymatic zone: Wide (12–15 mm.), hydrolysis of starch imperfect.
10. Gelatin, 18°, 35 days.
Growth: Cream-colored to brownish, spreading chiefly on side of tube.
Aerial mycelium: Abundant, white, covering all growth.
Soluble pigment: Brown in liquefied portion.
Liquefaction: Slow, presence of starch seems to further it.
11. Synthetic solution.
Growth: None.
12. Milk, 37°.
Growth (25°): Dark brown surface zone, abundant white aerial mycelium.
Coagulation: 6–7 days.
Peptonization: Begins soon after coagulation, advances very slowly, and is not completed in 50 days.
Change of reaction: Unchanged.
13. Glucose broth, 25°, 12 days.
Growth: Thin, dark gray pellicle on surface consisting of round colonies.
Aerial mycelium: None to a thin ash-gray layer.
Soluble pigment: Deep brown, spreading downward.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Fair, with glycerin as a source of carbon.
 2. *Proteolytic action*: Scant on milk, fair to good (in presence of 1 per cent starch) on gelatin.
 3. *Change of reaction*: Unchanged or slightly acid in gelatin, unchanged in milk, distinctly acid in glucose broth.
 4. *Inversion of sugar*: None.
 5. *Diastatic action*: Good on plate, chiefly to the erythro reaction.
 6. *Growth on cellulose*: None.
- Hab.* Hawaiian pineapple soil.

Actinomyces 206

I. MORPHOLOGY.

1. Spirals.
None on most media; the growth takes place in small individual clumps with straight, branching hyphae; a few long, open spirals present on the calcium malate agar.
2. Conidia.
Synthetic agar: Spherical and oval, 0.9 to 1.1 x 0.9 to 2.0 μ .

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.
Growth: Abundant, spreading, developing deep into the medium; color of growth yellow to olive-ocher (Rdg. XXX, 21"); reverse yellow to almost black.
Aerial mycelium: Mouse-gray to light drab (Rdg. XLVI, 17""–C).
Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Thin growth penetrating to some extent into the medium, edge myceloid, color deep colonial buff (Rdg. XXX, 21"b).

Aerial mycelium: Thin, over large part of surface; minute droplets of water forming a silvery zone on surface of it; color violet-gray (Rdg. LII, 59"") with yellowish margin.

Soluble pigment: None.

3. Glucose agar.

Growth: Abundant, restricted on surface and penetrating to some extent into the medium; center raised; edge entire; color as before.

Aerial mycelium: Covering only portions of surface, color as on calcium malate.

Soluble pigment: None in 15 days, in 35 days faint yellow.

4. Nutrient agar.

Growth: White smear, surface glistening.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood serum, 37°.

Growth: Spreading, glistening, cream-colored smear.

Aerial mycelium: None.

Soluble pigment: None.

Liquefaction: Medium, begins in 6-7 days.

6. Egg-media, 37°.

Growth: Thin, spreading, gray growth.

Aerial mycelium: Thin white patches.

Soluble pigment: None.

7. Starch plate.

Growth: Thin, spreading, yellowish green in color.

Aerial mycelium: Dark gray.

Enzymatic zone: Good (3).

8. Potato plug.

Growth: Abundant, much wrinkled, elevated, gray at first (4 days), later (8 days) turning sulfur-yellow on edge; in 30 days, the plug is nearly all destroyed (shrivelled up) by the growth of the organism.

Aerial mycelium: Abundant all over growth, light yellow with mouse gray patches.

Color of plug: Unchanged.

9. Carrot, 25°, 22 days.

Growth: Abundant, spreading, cream-colored.

Aerial mycelium: Abundant, powdery, gray colored, all over growth.

Color of plug: Unchanged.

10. Gelatin, 18°, 35 days.

Growth: Cream-colored flaky mass on bottom of liquefied portion; in exposed part, close to glass, yellow.

Aerial mycelium: Thin white on yellow exposed portion of growth.

Soluble pigment: None.

Liquefaction: Rapid (4 cm. in 35 days).

11. Synthetic solution.

Growth: Scant growth to almost none. When glycerin is substituted for saccharose there is formed an abundant yellowish brown pellicle on surface of liquid, with a yellow aerial mycelium and a yellow soluble pigment.

12. Milk, 37°.

Growth (25°): Faint pinkish growth, accompanied by hydrolysis of milk.

Coagulation: 4-5 days.

Peptonization: Begins in 4-5 days and proceeds very rapidly so that it is all completed in 10-12 days.

Change of reaction: Faintly (1) to fairly alkaline (2).

13. Glucose broth, 25°, 12 days.

Growth: Wide, thick ring on surface in contact with glass; color sulfur-yellow, often shade of orange is produced.

Aerial mycelium: Thin, ash-gray, all over growth.

Soluble pigment: None or faint sulfur-yellow pigment.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation*: Fair, with starch and glycerin as sources of carbon.

2. *Proteolytic action*: Very good in milk and glucose broth; excellent on gelatin, but only good in the presence of 1 per cent starch.

3. *Change of reaction*: Fairly alkaline in milk and glucose broth; distinctly alkaline in acid gelatin, in absence and presence of starch.

4. *Inversion of sugar*: Positive, often negative.

5. *Diastatic action*: Good, no starch left in a 1 per cent solution at the end of 14 days; good on plate, enzymatic zone 12-15 mm. wide.

6. *Growth on cellulose*: Good, with paper as the only source of carbon.

Hab. Isolated from Oregon, California adobe, and Maine Aroostook soils.

COMPARATIVE CULTURAL AND BIOCHEMICAL STUDIES

Many species have shown, in the cultural and biochemical studies, unique differences which assist in their separation, but the most important results are obtained when these organisms are separated into groups, which resemble closely one another in their metabolism. The actinomycetes vary in their metabolism as much as any other large complex group of forms of life, and a distinct separation into species is almost next to impossible, because new forms will be found which will tend to bring two distinct species together; it is therefore much more advisable, in the study of these organisms, to separate them into species-groups where the species resembling one another in their metabolism would fall. A number of the descriptions of the species given above are based not upon a single organism but upon a group of closely related forms.

Several simple synthetic media were used so that the information could be readily duplicated and a better insight into the metabolism of the organisms could be obtained. Many of the results reported were repeated two and three times, and any differences that occurred on the following observations were carefully noted. As stated above, most of these organisms were described 4 years ago, soon after their isolation from natural substrata; after they were grown for this period of time on artificial, usually synthetic, media, some organisms have shown cultural differences distinctive from those obtained 4 years previously, and these differences also were carefully noted. In inoculating cultures for the work, aerial mycelium (including conidial) was used where possible, otherwise a piece of the growth was transferred by means of a

sterile platinum loop or heavy needle, the point of which was flattened. Where an organism failed to grow on inoculation upon a certain medium, the inoculation was repeated on another tube or often on a fresh lot of medium. It is quite possible that even with these precautions certain differences escaped attention, but these would be usually of limited value in establishing the cultural identity of an organism, and it is believed that the cultural and biochemical studies reported represent fairly accurately the comparative reactions of the organisms used.

For convenient comparison of the species, the data are tabulated below. A complete discussion of the metabolism of actinomycetes, including complete tables for this group of organisms, has recently been published elsewhere (43). Only several of the tables are repeated here for purposes of comparison, but, for a complete historical review on the metabolism of these organisms and a discussion of the data, the reader is referred to the other papers.

UTILIZATION OF CARBON COMPOUNDS AS SOURCES OF ENERGY

In all the studies of utilization of carbon compounds, 3 per cent of the sources of carbon were added to the synthetic solution with 0.2 per cent of NaNO_3 as the only source of nitrogen.

Arabinose. Out of 23 organisms tested for the utilization of arabinose as the only source of carbon, *A. aureus*, *A. poolensis*, *A. albosporus*, *A. lipmanii*, *A. verne*, *A. bobili*, *A. reticulus-ruber*, *A. 128*, *A. 205*, *A. 168*, *A. asteroides* and *A. 104* refused to grow at all; *A. roseus* and *A. ruber* produced only a few flakes at the bottom of the tube; *A. violaceus-ruber* and *A. violaceus-caesari* a few minute colonies through the medium or on the surface; *A. albus* made a fair surface growth; *A. viridochromogenus*, *A. griseus*, *A. exfoliatus*, *A. fradii*, *A. scabies* and *A. diastaticus* made a good to very good surface growth with a fair to abundant aerial mycelium. The organisms that made a good growth affected a change in the hydrogen-ion concentration of the medium towards alkalinity. *A. violaceus-caesari* produced a blue soluble pigment; *A. griseus*, *A. scabies* and *A. exfoliatus* a yellow to brown soluble pigment. *A. violaceus-ruber*, *A. ruber*, *A. roseus* and *A. viridochromogenus* reduced the nitrate to nitrite in appreciable amounts, while some cultures (*A. fradii*, *A. lipmanii*) contained mere traces of nitrites (*A. madurae*, *A. hominis* and *A. bovis* were used only in a few instances in the carbon studies).

Dextrose. Dextrose proved to be one of the best sources of carbon for the actinomycetes. Out of the 26 organisms inoculated upon the solution, only *A. verne*, *A. poolensis*, *A. 205* and *A. 104* made a scant flaky growth through the medium or on the bottom, with no change of reaction or nitrite production (traces with *A. verne*). Most of the other organisms (*A. violaceus-ruber*, *A. exfoliatus*, *A. 168*, etc.) made a very good to excellent growth, usually in the form of surface colonies or in the form of a heavy pellicle, often developing out of the colonies growing together, and a good characteristic, aerial mycelium.

A. griseus and *A. 168* produced a golden pigment, while *A. viridochromogenus*, *A. scabies* and *A. exfoliatus* produced a brown soluble pigment. The change of reaction was distinctly towards the alkaline for the organisms that produced a good growth. *A. violaceus-ruber*, *A. roseus*, *A. reticulus-ruber* and *A. asteroides* produced maximum quantities of nitrites, a few other species only traces or fair quantities, while most organisms did not produce any nitrite in the presence of dextrose.

Maltose. Maltose was found to be also a very good source of carbon. Out of the 26 organisms studied, only *A. poolensis*, *A. albosporus*, *A. madurae* and *A. bovis* made a scant flaky growth on the bottom of the tube. Most of the organisms made a good to very good growth with this source of carbon, but only *A. viridochromogenus* made an excellent growth, accompanied by a distinct change in reaction towards acidity. The growth is usually on the bottom of the tube, but quite often (*A. griseus*, *A. diastaticus*, *A. 168*) on the surface of the liquid with an abundant aerial mycelium. The change in reaction was in a few cases toward acidity (*A. viridochromogenus*, *A. violaceus-caesari*, *A. albus*, and *A. 104*), in most cases unchanged or changed to distinctly alkaline (P_H changed for *A. violaceus-ruber* from 6.2 to 7.2, *A. griseus* to 7.6, *A. diastaticus* to 8.0, *A. hominis* to 7.4, *A. reticulus-ruber* to 7.1, *A. 205* to 7.2, *A. ruber* to 7.0.) The nitrite production was good for a number of organisms, *A. violaceus-ruber* producing a maximum quantity.

Saccharose. Saccharose is a rather poor source of carbon for many actinomycetes, because not all the species are able to invert this sugar. It is quite possible that some organisms, on continued cultivation on artificial media, begin to produce some inverting enzyme, as was observed in a few cases. The growth in solution was usually scant to fair, in the form of flakes on the bottom of the tube or as colonies throughout the medium; only *A. ruber* and *A. exfoliatus* produced an excellent growth on saccharose in solution. The change of reaction was either faintly acid (*A. asteroides*, *A. scabies*, and *A. exfoliatus*), faintly alkaline or distinctly alkaline (*A. griseus*, *A. diastaticus*, *A. aureus*, *A. reticulus-ruber*, *A. madurae*, *A. bobili*, *A. viridochromogenus* and *A. 104*). The reduction of nitrate to nitrite is usually none or scant, excellent only for *A. violaceus-ruber* and *A. bovis*, and good for a few others (*A. asteroides*, *A. verne*, *A. albus*, and *A. reticulus-ruber*). Of course these comparisons hold true, in the case of this source of carbon as well as the others, only in liquid media; on agar media much better growth of most species is obtained.

Lactose. Lactose is a good source of carbon for most species. Out of nearly 30 species tested, only *A. violaceus-caesari* and *A. 104* produced a scant flaky growth on the bottom of the tube and only *A. violaceus-ruber* an excellent growth. Most organisms produced a good to very good growth,* usually in the form of colonies on the surface of the liquid or in the form of a heavy pellicle accompanied by fair to abundant aerial mycelium. *A. scabies*, *A. exfoliatus* and *A. viridochromogenus* produced a brown soluble pigment and *A. violaceus-ruber* a blue pigment. The change of reaction was usually toward

alkaline. Most organisms produced no nitrite or mere traces; only *A. bovis* and *A. violaceus-ruber* produced a maximum quantity, *A. verne* and *A. viridochromogenus* "very good" amounts of nitrites.

Glycerin. Glycerin favored a good development of most species, although fewer organisms made as abundant a growth as on dextrose and starch in solution. It is interesting to note that *A. poolensis* that made a scant growth on most other carbon compounds made a very good growth on glycerin. *A. verne*, *A. 128*, *A. albosporeus*, *A. 104* and *A. 205* produced only a scant growth. *A. griseus*, which made a heavy growth on most carbon compounds, except saccharose and the salts of organic acids, made a rather limited growth on glycerin. A soluble pigment was formed only in the culture of *A. exfoliatus*. The reaction of the solution was either left unchanged or changed to faintly acid or alkaline; *A. asteroides* and *A. violaceus-ruber* caused a distinct change in the glycerin medium toward acidity. Nitrites were produced by most organisms, but in quantities not larger than mere traces; only *A. violaceus-ruber*, *A. viridochromogenus*, *A. lipmanii*, *A. bovis*, *A. hominis* and *A. asteroides* produced appreciable quantities of nitrites.

Mannite. Mannite was found to be a good source of carbon only for about half of the actinomycetes tested. *A. aureus* was the only species that made an excellent growth, while *A. poolensis*, *A. violaceus-caesari*, *A. 128*, *A. roseus*, *A. reticulus-ruber*, *A. bobili*, *A. asteroides*, *A. 104*, and *A. 205* made a scant growth. The hydrogen-ion concentration was left unchanged or usually changed to alkaline, notably *A. griseus* changing the reaction from P_H 7.2 to 7.7, *A. scabies* to 7.6 and *A. luteus* to 7.5. Very few cultures changed the medium to slightly acid; among these *A. asteroides* occupies the most prominent place, changing the P_H values from 7.2 to 6.5. A soluble brownish pigment was produced only by *A. exfoliatus* and *A. viridochromogenus*. The reduction of nitrates to nitrites was scant or entirely absent for most organisms; *A. violaceus-ruber*, *A. reticulus-ruber*, *A. verne* giving the maximum quantities, while *A. albosporeus* and *A. griseus* gave only good reduction.

Starch. Starch was found to be the best source of carbon of all the compounds tested for most of the 26 species used. Only *A. verne* and *A. 104* that made a rather scant growth on all other carbon sources gave a scant flaky growth on starch as the only source of carbon and *A. asteroides* made hardly any growth at all; most other organisms made a good to very good growth usually in the form of colonies or a heavy pellicle on the surface of the liquid and accompanied by a good aerial mycelium. The change in reaction of the medium was usually none or (more often) toward distinct alkalinity. So, for example, *A. ruber* changed the hydrogen-ion concentration of the medium (P_H values) from 7.0 to 7.6, *A. diastaticus*, *A. albosporeus* and *A. viridochromogenus* to 7.5, etc. Some few organisms produced a faint acidity in the medium, *A. 128* and *A. 104* changing the P_H values from 7.0 to 6.8. A brown soluble pigment was produced by *A. lipmanii* and *A. exfoliatus*, while a fair brown (golden) pigment by *A. 128*, *A. griseus*, *A. fradii*, *A. 168* and

A. 205. The reduction of nitrates to nitrites was scant or none for most of the species that gave weak nitrite accumulation with other carbon compounds, but the active nitrate-reducing organisms usually allowed large quantities of nitrites to accumulate in the medium. The high average for nitrate reduction with starch as a source of carbon should not therefore be looked upon as an indication that more organisms reduce nitrates with this source of carbon than with any other, but that those that reduce nitrates readily do it even more actively with starch as a source of carbon, which as was shown elsewhere (43) is explained by the metabolism of the organisms. Starch as a good source of energy allows a better development of the organisms and this is accompanied by a greater reduction of nitrates in the medium. *A. violaceus-ruber*, *A. roseus*, *A. lipmanii* and *A. reticulus-ruber* gave the "excellent" reduction of nitrates.

Starch is readily utilized by most actinomycetes because most of them produce an active diastatic ferment which hydrolyzes the starch actively, thus making it readily available as a source of carbon. This was shown to hold true both by the plate and the tube methods, as seen in table 7. A complete discussion on the hydrolysis of starch by actinomycetes will be found in the paper on the "Carbon Metabolism of Actinomycetes" (43).

Inulin. Out of 12 species tested with this source of carbon, only *A. reticuli* made a scant growth, while *A. ruber* and *A. bovis* made a good to very good growth. The other 9 species made only a fair growth; this was usually in the form of colonies on the bottom of the tube or a thin pellicle on the surface, covered with a thin aerial mycelium. With NaNO_3 as a source of nitrogen, 9 out of the 12 cultures changed the medium from nearly neutral (P_H 6.8) to distinctly alkaline (P_H 7.6-8.1), only *A. exfoliatus*, *A. ruber*, and *A. diastaticus* produced no change in reaction of faint acidity. Only *A. exfoliatus* produced a yellowish soluble pigment.

Cellulose. The utilization of cellulose by actinomycetes was tested by several different methods, but none of them proved very satisfactory, as was shown above. This can be further emphasized by the fact that the different methods did not check up with one another; some organisms that were found to use cellulose readily by one method were not found to do so by another; this is no doubt due to the difference in the form of cellulose used in the different methods. The simplest and easiest way of testing the cellulose utilization by actinomycetes is the introduction of a piece of filter paper into the synthetic solution, without any other source of carbon. Those organisms that can use cellulose as the only source of carbon will be found to form colonies or flaky growth on the paper and then throughout the medium; this is also accompanied by a change in the reaction of the medium and the reduction of nitrates to nitrites. As was shown elsewhere (43), the reduction of nitrates and the changes in the hydrogen-ion concentration of the medium can be taken, for many organisms, as an index of their utilization of the proper carbon compound; this is particularly important in the study of the cellulose utilization by

the actinomycetes as the only source of carbon, since not only the actual growth obtained, but also the change in the hydrogen-ion concentration and the reduction of nitrates can be recorded. Out of the 30 or so species studied on cellulose, many produced a scant flocculent growth on the bottom of the tube or none at all. *A. violaceus-ruber*, *A. violaceus-caesari*, *A. scabies*, *A. ruber*, and *A. 205* made a fair growth accompanied by a faint change in reaction toward alkalinity and traces of nitrites in some instances (fair with *A. violaceus-ruber*); *A. verne*, *A. 168* made a good growth, *A. bobili* and *A. reticulus-ruber* made a very good growth. *A. asteroides*, with a scant growth, was the only organism that changed the reaction of the medium to distinctly acid (from P_H 7.0 to 6.5). *A. ruber*, *A. albus*, *A. exfoliatus*, *A. aureus*, *A. violaceus-ruber*, *A. scabies* and *A. bobili* made a very good growth on the cellulose plate, most of them producing a clear ring around the colony; *A. albus*, *A. ruber*, *A. viridochromogenus* and *A. lipmanii* made the best growth upon filter paper by using the method of Krainsky (22).

Organic acids. Several sodium salts of organic acids were studied as sources of carbon for actinomycetes (30 gm. per liter), namely, the salts of acetic, lactic, malic, tartaric and oxalic acids. The growth of most of the organisms on these acids was limited to a few flakes on the bottom of the tube, accompanied in most instances by a distinct alkalinity and none or only scant nitrate reduction. The alkalinity was higher with these carbon compounds than with the previous ones, due to the fact that, in addition to the alkalinity derived from the nitrogen source, the acid radical of the carbon compound was used as the source of energy and the cation probably left in the medium, forming carbonates, thus resulting in distinctly alkaline reaction. *A. bobili*, *A. reticuli*, *A. reticulus-ruber* and *A. ruber* made a fair growth on the acetic acid, while *A. violaceus-ruber*, *A. griseus*, *A. asteroides*, *A. bovis*, *A. reticulus-ruber* and *A. ruber* produced, with this carbon source, more than scant quantities of nitrites. *A. viridochromogenus*, *A. madurae*, *A. asteroides*, *A. albus*, and *A. 168* made more than a scant growth (*A. asteroides* best) on the lactic acid, while only *A. asteroides* produced more than scant quantities of nitrites in the medium, with this source of carbon. *A. griseus* and *A. viridochromogenus* produced more than a scant growth on malic acid, while *A. violaceus-ruber*, *A. griseus*, *A. asteroides* and *A. 205* allowed a good accumulation of nitrites in the medium. *A. viridochromogenus*, *A. asteroides*, *A. exfoliatus* and *A. 168* produced a fair growth on tartaric acid, while only *A. asteroides* allowed a fair accumulation of nitrites with this source of carbon. No organism made more than a scant growth on oxalic acid, while *A. violaceus-ruber*, *A. reticulus-ruber* and *A. hominis* allowed a good to fair nitrite accumulation. This will tend to show that the organic acids form (as sodium salts), with very few exceptions, rather poor sources of carbon for the actinomycetes (succinic acid also gave similar results). Some acids (malic) were offered in the form of ammonium salts and seemed to be used much more readily than the sodium salts. This may be due to the fact that, in the case of the latter, the medium becomes so alkaline that the organisms cannot readily grow further.

UTILIZATION OF NITROGEN COMPOUNDS

The following organisms were used for this study: *A. violaceus-ruber*, *A. griseus*, *A. aureus*, *A. bobili*, *A. scabies*, *A. albus*, *A. viridochromogenus*, *A. verne*, *A. ruber*, *A. bovis*, *A. asteroides* and *A. reticuli*. Three per cent of glycerin was added (in place of saccharose) to the synthetic solution as the source of carbon; glycerin was used, because, as was shown above, it is readily assimilated by most organisms, and, if it does not allow as abundant a growth of some species as does starch or dextrose, it never allows as poor a growth as the organic acids, arabinose or saccharose; another advantage in the use of glycerin is the fact that the reaction of the medium does not change appreciably on sterilization, as do the media containing dextrose, lactose or maltose. The nitrogen compounds were studied only as sources of nitrogen and not of carbon, which was possible in the presence of a good source of carbon, such as glycerin. These compounds were added to the synthetic solution, the organic compounds 0.5 per cent, the inorganic salts 0.2 per cent. The cultures were grown in duplicates and incubation took place at 25° for 15 and 30 days and, in the case of *A. bovis* and *A. asteroides* for 30 and 60 days. All these studies, as well as the experiments on the utilization of carbon compounds, were carried on in solution; had a solid medium been used, a better growth might have been obtained with the poorer sources of carbon and nitrogen.

Sodium nitrate. Sodium nitrate was used as the only source of nitrogen in the previous investigations on the utilization of carbon compounds. *A. violaceus-ruber*, *A. bobili*, *A. griseus*, *A. scabies*, *A. verne*, *A. bovis*, *A. asteroides* and *A. reticuli* produced only a scant to fair growth on this medium. *A. viridochromogenus* and *A. ruber* produced a fair growth, *A. aureus* and *A. albus* an excellent growth in 30 days. The reaction was changed in nearly all cases to alkaline, only *A. violaceus-ruber* changing it to slightly acid and *A. asteroides* to distinctly acid. Few actinomyces produce a very heavy growth with NaNO_3 as a source of nitrogen and glycerin as a source of carbon, many produce a fair to good growth and many a scant growth in solution. When agar is added to the medium, the growth is much heavier.

Sodium nitrite. Those organisms that use well sodium nitrate as a source of nitrogen, will grow readily on sodium nitrite, with very few exceptions, especially if the latter is present in low concentrations (0.02 per cent or less); *A. aureus*, *A. violaceus-ruber*, *A. scabies* and *A. viridochromogenus* made a good growth while the other species grew only scantily. *A. violaceus-ruber*, *A. aureus*, *A. viridochromogenus*, *A. scabies*, *A. verne*, and *A. asteroides* changed the reaction of the medium to acid, while the others did not produce any change in reaction or made the medium faintly alkaline. *A. scabies* and *A. viridochromogenus* produced a soluble brown pigment, *A. griseus* a yellowish and *A. violaceus-ruber* a purple pigment. The amount of growth on NaNO_2 is not so abundant as on NaNO_3 , due to the fact that NaNO_2 , in too large concentrations, exerts a toxic effect upon the organism. Although, in this experiment,

only 0.2 per cent of NaNO_2 was used, it is quite possible that for many organisms, even this concentration is toxic. If the concentration of NaNO_2 is reduced too much, it is soon exhausted by the organism. There is no doubt that the organisms that reduce nitrates actively will grow readily with NaNO_2 as a source of nitrogen, when present in not too large concentrations; while those species which do not reduce the nitrates appreciably did not make much of a growth on NaNO_2 , in the concentrations studied. By varying the concentration of the NaNO_2 from 5 to 500 mgm. per 100 cc. of solution, it was found that most species grew readily in the low concentrations, using up all the nitrite in a few days; with the increase in concentration of the NaNO_2 , the toxicity was increased and, at the highest concentration, most species refused to grow at all, while few made some growth. Thus the different species vary in their ability to withstand higher concentrations.

Ammonium salts. Ammonium carbonate and ammonium sulfate offer rather poor sources of nitrogen to the actinomycetes. Only *A. aureus* made a fair growth on the first and an excellent growth on the second in 30-40 days at 25°. *A. ruber* made a scant growth on the carbonate and a fair growth on the sulfate. All the other species studied made none or only a very scant growth on these compounds with glycerin as a source of carbon. With dextrose the growth on the ammonium salts as sources of nitrogen was much more abundant. Only *A. reticuli* made no growth on the sulfate and a scant growth on the carbonate and *A. madurae* grew scantily on the first and made no growth on the second with dextrose as a source of carbon. The only organism that grew well (good) on the sulfate was *A. scabies*, all the others making a scant to fair growth on it. With ammonium carbonate as a source of nitrogen and dextrose as a source of carbon, *A. aureus* and *A. violaceus-ruber* made a very good growth, *A. asteroides*, *A. viridochromogenus*, *A. verne* and *A. scabies* made a good growth, and *A. bovis* and *A. asteroides* gave only a fair growth. In the presence of ammonium sulfate, the reaction in all cases became acid, both with glycerin and dextrose (P_H changing from 5.8 to 4.6-4.2 with dextrose). The same thing holds true for the ammonium carbonate (*A. aureus* changing P_H from 6.8 to 4.4), only *A. madurae*, *A. bovis*, and *A. reticuli* changed the reaction in the presence of the carbonate to slightly alkaline. The limited growth of the organism on the ammonium sulfate medium and to some extent also on the carbonate is no doubt due to the fact that it uses up the cation as the source of nitrogen, leaving the anion in the medium. This results, in a medium rather poorly buffered (only 0.1 per cent K_2HPO_4), in a distinct change in reaction, so that it soon becomes distinctly acid and below the maximum acidity at which the organisms can grow. It was pointed elsewhere and will also be shown in another connection in this paper that the limiting P_H (on the acid side) for the growth of the actinomycetes is 4.8-5.2. In the case of ammonium sulfate and carbonate media, the reaction was changed, in most instances, to P_H 4.2-4.6, which falls below the maximum acidity that the organisms can tolerate, this leading to a cessation of growth. When the ammonium

is offered in a form where the acid radical is used up as well (ammonium malate), the amount of utilization of this source of nitrogen is greatly increased.

Urea and acetamide. Urea and acetamide allowed only a scant growth of most actinomycetes, with glycerin as a source of carbon. *A. violaceus-ruber*, *A. aureus* (acetamide only), *A. bobili* (acetamide only), *A. scabies*, *A. ruber*, and *A. viridochromogenus* (urea only) made a fair to good growth. The reaction was either unchanged or changed very faintly to acid or alkaline. The utilization of urea, as a source of nitrogen, was tested with dextrose as a source of carbon. Most organisms made here also only a faint growth, but *A. violaceus-ruber*, *A. aureus*, *A. viridochromogenus* and *A. asteroides* made a very good to excellent growth. The change in reaction was in all cases to alkaline (P_H changed from P_H 7.4 as high as P_H 8.6), except *A. viridochromogenus*, which left the medium slightly acid. The utilization of a definite nitrogen source depends in many cases on the source of carbon. The amides, which form rather poor sources of nitrogen for the group as a whole, with very few exceptions, and are entirely valueless as sources of carbon, will show distinct variation depending on the source of carbon; in the presence of a good source of carbon, some species may make a very good growth; this fact can help a great deal in the separation of the different organisms.

Glycocoll. Glycocoll forms a good to very good source of nitrogen. *A. bovis* and *A. asteroides* were the only two species which did not make any more than a fair growth in 30 days at 25°. An abundant growth was accompanied by a distinct decrease in the amino nitrogen content of the medium, while a slight growth resulted only in a very small decrease. This fact can be used to advantage to measure the utilization of the amino acids by microorganisms and can be taken, to a certain extent, as an indication of the growth made. *A. aureus*, *A. viridochromogenus* and *A. reticuli* produced a brown pigment, while the pigment produced by *A. griseus*, *A. albus*, and *A. bovis* was yellowish, and that of *A. violaceus-ruber* red, in 30 days. The reaction was changed in most instances to alkaline, except *A. violaceus-ruber* (P_H changed from 7.1 to 6.4 in 15 days and 5.6 in 30 days) and *A. asteroides* (P_H from 7.1 to 6.4 in 30 days). Most organisms produced slight quantities of ammonia from glycocoll.

Leucin. Leucin, as well as asparagin and glycocoll, offer good sources of nitrogen for actinomycetes. *A. bobili*, *A. scabies*, *A. ruber*, *A. viridochromogenus* and *A. reticuli* produced soluble brown pigments, that of *A. aureus* and *A. griseus* was yellowish and of *A. violaceus-ruber* red. These same pigments were produced by these organisms on asparagin. The utilization of the nitrogen in leucin, as well as in glycocoll and asparagin, is measured by the decrease in the amino nitrogen content of the medium. Only *A. scabies*, *A. bovis* and *A. asteroides* produced traces of ammonia in the leucin cultures. The interesting point to be noticed here is the decrease of the hydrogen-ion concentration of the cultures (the reaction becoming always more or less acid, P_H was changed from 7.3 by *A. scabies* to 7.1 in 30 days and *A. violaceus-ruber* to 5.6 in same period, all other species falling between these two).

Asparagin. Only *A. bobili* and *A. asteroides* grew scantily on this source of nitrogen and all the other species made a fair to excellent growth. Ammonia was produced readily by most species in appreciable amounts, so that this compound can be used more readily than the other two for testing the ammonia-producing power of actinomycetes. The reaction was changed in most cases to alkaline, except *A. violaceus-ruber*, *A. aureus*, *A. viridochromogenus* and *A. asteroides* that changed it to acid.

Asparagin, as well as the amino acids, is used readily as a source of carbon and nitrogen. In the absence of any other available source of carbon the organisms grew very readily on asparagin, which served as a source of both carbon and nitrogen.

Fibrin, casein, egg-albumin and peptone. Cultures containing proteins or peptone as sources of nitrogen allow a maximum growth of actinomycetes. Only *A. bobili* and *A. reticuli* produced a scant growth on egg-albumin in 15 days, the growth becoming good in 30 days; otherwise it was chiefly good or very good. Most of the species studied produced a yellow to brown soluble pigment, except *A. violaceus-ruber*, which produced a red or blue pigment depending on the reaction of the culture; *A. verne* produced a brownish pigment only on peptone, *A. albus*, a yellowish pigment on fibrin and casein and *A. asteroides* no pigment at all. The reaction was changed on fibrin, in most cases, to acid, except *A. bovis*, *A. verne*, *A. ruber* and *A. scabies*; on casein to acid, with the same exceptions, including *A. bobili*; on peptone to acid with the same exceptions as on fibrin, also *A. albus*; on egg albumin the reaction of the cultures studied was changed to acid, except *A. griseus*, *A. ruber* and *A. bobili*, *A. scabies*, *A. verne*, *A. bovis*, and *A. reticuli*, which left the reaction unchanged or made the medium more alkaline. These four organisms, *A. bovis*, *A. verne*, *A. scabies* and *A. ruber* changed the reaction of the cultures containing different proteins or peptone always to alkaline, while other organisms such as *A. violaceus-ruber*, *A. asteroides*, *A. aureus*, *A. viridochromogenus* changed the same media always to acid. The proteins and peptone were hydrolyzed to some extent by the organisms (directly or by means of enzymes) to amino-nitrogen rich compounds, although no general conclusion can be drawn here, since some species affected a greater splitting of one protein and others of another. Ammonia was readily produced by different organisms from the different proteins.

Casein, fibrin, asparagin, and other proteins and amino acids can be used as sources of both carbon and nitrogen. Most species grew very rapidly on these proteins and amino acids, in the absence of any other carbon compounds, deriving from them both their energy and their nitrogen supply. The reaction usually becomes alkaline probably because of the accumulation of ammonia in the medium. The amino-nitrogen content of the media containing the proteins increases considerably and decreases in the media containing the amino acids, which is readily explained by the metabolism of these organisms.

Creatinine. All species tested produced a "fair" to "good" growth in solution on creatinine as the only source of nitrogen. The reaction of the medium

was changed in most cases to faintly acid; *A. 205* produced a characteristic yellow pigment; *A. violaceus-ruber* a bluish and the few chromogenus species a brownish pigment.

Tyrosin. The utilization of tyrosin as a source of nitrogen was tested both on agar and in solution, with saccharose and glycerin as sources of carbon. Most species grew readily on agar, but only *A. scabies* and *A. 205* produced a soluble brown pigment, indicating that only these two species produced tyrosinase. Not all the strains of *A. scabies* produced the brown pigment on tyrosin; the strains isolated by the writer did it readily, while those obtained from other sources, particularly when grown for several years on artificial media, seem to have lost that property or produced only a faint brown pigment. When all the species that produced soluble brown pigments on organic media were tested in tyrosin solution (synthetic solution containing glycerin and free from any other sources of nitrogen), *A. scabies* produced a deep brown color, *A. viridochromogenus*, *A. pheochromogenus* and *A. 205* produced faint brown pigments while *A. violaceus-ruber* produced a soluble blue color. The growth of all the species was fair to good.

We can thus readily see that the production of a brown pigment on protein media is not a result of the action of tyrosinase alone, since this pigment is formed only by few species from tyrosin, while several other species are able to produce a soluble brown pigment on tyrosin-free media, such as the media containing the other amino acids, inorganic nitrogen salts and proteins free from tyrosin. The only conclusion that we can make here would be that, as Beijerinck (3) suggested, tyrosinase is a mixture of two enzymes, both of which are produced by *A. scabies* and the other species producing the pigment on tyrosin; the other species producing pigments on other amino acids and proteins are able to produce oxidases which can form brown pigments from organic substances free from tyrosin. This fact can be further emphasized by the fact that a few species can produce soluble pigment including brown (*A. exfoliatus*) on inorganic media, free from any traces of proteins or amino acids.

Aerial mycelium was formed by most of the species tested on the different nitrogen media, except *A. verne* and *A. bobili*, which did not form any aerial mycelium on all these media; *A. scabies* produced aerial mycelium only on casein and *A. bovis* on casein and egg-albumin.

Milk (table 3). The milk cultures of the actinomycetes present interesting differences, these depending on the proteolytic powers of the species. A complete discussion of this subject will be found elsewhere (43).

The chemical changes produced in milk due to the action of actinomycetes can be used in the identification of the different species. One group of organisms, including *A. griseus*, *A. poolensis*, *A. madurae*, *A. ruber*, and *A. 206*, which coagulated the milk in 3-5 days, soon begin to peptonize the curd, and have it all digested in about 10 days. The amount of amino-nitrogen and ammonia produced by these organisms is very great, as for example, *A. griseus* converted in 15 days 57.7 per cent, *A. madurae* 59.8 per cent and *A. poolensis*

34.6 per cent of the nitrogen of the milk into amino nitrogen. *A. griseus* converted 23.3, *A. poolensis* 21.8, *A. ruber* 20.1 and *A. 206*, 23.0 per cent of the nitrogen of the milk into ammonia in 40 days. The reaction of the milk was changed by these species to the most alkaline.

The second group of organisms contains those species that coagulate the milk rapidly, but peptonize the curds slowly, so that in most cases the curd is not digested even in 45 days. We would include in this group *A. reticuli*, *A. reticulus-ruber*, *A. rutgersensis*, *A. 161* and *A. 145*. It would seem that these organisms produce as strong a rennet-like enzyme as the first group of organisms, but a weaker proteolytic enzyme, which therefore accounts for the slow peptonization of the curd. These species are further characterized, in most instances, by the fact that the reaction of the milk is either unchanged as in the case of *A. reticuli* and *A. 145*, or made only faintly alkaline.

Several species, namely *A. 128*, *A. hominis*, *A. viridochromogenus*, *A. diastaticus*, and *A. verne* stand between the two groups, producing a rapid coagulation, while the peptonization is more slow than for group 1 and more rapid than for group 2.

A. fradii, *A. lipmanii*, *A. bovis*, *A. scabies*, *A. 96*, *A. citreus* and *A. luteus* coagulate the milk only in 10-12 days, followed by a rather rapid peptonization. These organisms are characterized by an alkalinity lower than that of group 1 but higher than that of group 2, no doubt due to the fairly rapid digestion of the clot.

A fourth group would comprise those species which do not coagulate the milk, but hydrolyze it without previous coagulation. Here we would include *A. albus*, *A. exfoliatus*, *A. violaceus-ruber*, *A. bobili*, *A. lavendulae*, *A. roseus* and *A. alboflavus*. These are all characterized by a very high alkalinity. It is very possible that the lack of coagulation is not due to the lack of a rennet-like enzyme, but merely to the fact that the digestion of the casein of the milk proceeds rapidly at its early stages, so that coagulation is not observed or is absent. That some of the organisms that coagulate the milk may produce hydrolysis without clotting can also be concluded from the fact that a few cultures (*A. citreus*, *A. 168* and *A. 205*) produced hydrolysis of the milk in some cases while in other cases the same species produced a clot and then digested it.

Some of these species included in this group were found, on further study, to be able to produce a clot in the milk under certain conditions, depending on the temperature of incubation, amount of inoculum and mother culture. The effect of environmental conditions on the metabolism of actinomycetes is taken up elsewhere (43). Finally we find a few species that seem to have no visible action upon the milk, although some digestion of the milk proteins has taken place; *A. aureus* and *A. asteroides* would belong to this group. The reaction of the milk became in most instances alkaline and in no case acid, although several species produced no change in the reaction of the milk.

37°

The cultures reported above were all incubated at 37°; at this temperature there is hardly any visible growth, although the action on the milk is very definite. At 25° most species readily produce on milk a visible growth accompanied by characteristic reactions, but these reactions are, for many species, not the same as those obtained at 25°. A glance at table 3 reveals this fact. The difference in reaction upon the milk at 25° and 37° is explained by difference in growth, enzyme production and activity of the enzymes at the two different temperatures. So, for example, *A. madurae* and *A. hominis* which grow more readily at the higher temperature, will produce at that temperature a more rapid clot formation followed by the peptonization of the clot; *A. laven-dulae*, which grows more readily at the lower temperature, will hydrolyze the milk in a shorter period of time. The changes in reaction, with very few exceptions, run parallel at both temperatures, although for 37° only an approximate change is given while for 25° the exact reaction is reported. The species belonging to the *chromogenus* groups possess a rather weak proteolytic power. All these species, when grown on milk at 25°, produced a surface brown to black ring accompanied by an imperfect clot formation and scant digestion; the amino-nitrogen content of the milk was rather low and the reaction quite alkaline (++ to +++).

Gelatin (table 4). Fifteen per cent gelatin in distilled water offers a good medium for the study of actinomycetes. All the species, with the exception of *A. asteroides*, liquefied the gelatin in 30-40 days at 18°. But the rapidity of liquefaction and the production of a soluble pigment are characteristic of the species, although these, particularly the rapidity of liquefaction, may change to some extent on continued cultivation upon artificial culture media. The rapidity of liquefaction is also influenced by the temperature of incubation. If the reaction of the gelatin is not adjusted, the culture becomes in most cases more alkaline, with very few exceptions. The gelatin is hydrolyzed, as a result of the growth of the organisms, with an increase in the amino nitrogen content of the medium. On the addition of 1 per cent starch to the gelatin, the latter is usually split to a smaller extent, while the hydrogen-ion concentration of the culture is usually greater (more acid) than in the absence of starch. Nearly all the species are able to utilize gelatin as a source of both carbon and nitrogen. In the presence of an available carbohydrate, the gelatin is used only as a source of nitrogen and therefore is hydrolyzed to a lesser extent.

Glucose broth (table 4). The ordinary glucose broth offers a very good medium for the cultivation of most species, although the growth itself is not characteristic. The growth is usually accompanied by an increase in the amino nitrogen content of the medium, a change in the hydrogen-ion concentration, depending on the initial reaction, and by the production of a soluble pigment.

THE REACTION OF THE MEDIUM

A detailed study on the influence of reaction upon the growth of actinomycetes and the change of reaction of culture media has been published elsewhere (43). The reaction was determined as the hydrogen-ion concentration measured by the hydrogen electrode or phenol-sulphone-phthalein series of indicators. The limiting hydrogen ion concentrations are about P_H 5.0 and P_H 9.0 for the group as a whole, although several species (*A. reticulus-ruber*) are able to grow at higher hydrogen-ion concentrations (P_H 4.6–4.8); the optimum reaction is at P_H 7.0–7.8. In most cases, if the initial reaction of the medium is too acid, it is made, in the course of the growth of the organisms, more alkaline, tending to bring the reaction toward the optimum; if the reaction of the medium is too alkaline, it is made more acid, also tending toward the optimum. So we find that gelatin having a P_H equal to 6.2 and milk of a similar reaction are changed in the majority of cases to a more alkaline reaction; while glucose broth which is originally alkaline (P_H 7.9) is changed, in the majority of cases, to a more acid condition. But this is not the rule, since several species are able to leave even distinctly acid media unchanged or make them even slightly more acid.

The change of reaction of the medium depends on the nitrogen source and the amount of buffer in the medium. When ammonium sulfate is present and the medium is rather poorly buffered (only 0.1 per cent K_2HPO_4) the medium soon turns distinctly acid (P_H 4.4–4.8), stopping any further growth of the organisms. The same holds true for other salts of ammonia, where the acid radical is not used up at the same time. In the case of ammonium salts of organic acids, such as ammonium malate, where the ammonium is used as a source of nitrogen and the malate as the only source of carbon, the reaction always changes to alkaline due to the fact that the malate is used up more readily as a source of energy than the ammonia as a source of nitrogen, the energy requirement of the organisms being greater than that of nitrogen.

Ammonium carbonate is used also much more readily than the sulfate, because the hydrogen-ion concentration is increased, in most cases, to a smaller extent. This is readily explained, since carbonic acid is much less ionized than sulfuric acid (or hydrochloric), hence we would expect that the ammonium salts of a weakly dissociated acid, even if the acid radical is not used by the organisms, would offer better sources of nitrogen than the salts of strong acids, because the latter will make the reaction of the medium distinctly more acid, unless a large amount of buffer is present.

When sodium nitrate is present as the only source of nitrogen the reaction is changed, in most cases, with nearly all sources of carbon to more alkaline. With sodium nitrite, the reaction is changed, in most cases, to more acid. This is explained (43) by the assumption that most species reduce the nitrate to nitrite and use the nitrogen in that form; in the process of reduction the hydrogen tension of the medium would be decreased, this tending to a lower hydrogen-ion concentration or a more alkaline reaction.

In the presence of organic nitrogenous substances, the medium may become either alkaline or acid depending on the initial reaction of the medium and the organisms. When the medium, richly buffered with proteins, such as milk, gelatin, glucose broth, etc., is acid to start with, it will become in most instances, more alkaline; when it is alkaline to start with, it tends, in many instances, to become more acid. The presence of certain nitrogenous substances, such as leucin, tyrosin, creatinine, tend to leave the medium always more acid, to a greater or less extent, at least with certain carbohydrates. Some species (*A. scabies*) tend to turn the medium, in the presence of proteins or peptones always more alkaline, other species (*A. asteroides*, *A. 145*) usually make the reaction of the same media more acid. It should be noted here that any figure in the paper enclosed in parentheses refers to the last word only.

INFLUENCE OF TEMPERATURE

The influence of temperature upon distribution and growth of microorganisms is an important factor in the study of a particular group. It was first of all important to find out the maximum and minimum temperatures for the growth of actinomycetes, and then the temperatures which destroy the species studied. Glucose broth was used for the cultivation of the species. Most organisms were found to grow readily at temperatures ranging from 15° to 37°, the higher temperature favoring a more rapid growth, so that a certain species would require 10-15 days to give the same amount of growth at 15-18° as it would give at 37° in 24-48 hours. Particularly is this true of *A. madurae*, *A. hominis* and *A. bovis*, which grew readily at the higher temperature and very slowly at the lower temperature. At 40°, *A. bovis*, *A. scabies*, *A. ruber* and *A. violaceus-ruber* made a scant and *A. griseus* a fair growth. At 45° none of these five organisms made any growth at all.

A. griseus, *A. bovis*, *A. scabies*, *A. ruber* and *A. violaceus-ruber* were grown on glucose broth at 25° for 8 days. The cultures were then kept for 1 hour at 70°, 75°, 80° and 90°; and then tested for sterility by inoculating from the glucose broth upon glucose agar and nutrient agar. All the 5 cultures survived 70° and 75° for 1 hour. *A. bovis* was killed and the other 4 species survived a temperature of 80° for 1 hour. Only *A. griseus* survived a temperature of 90° for 1 hour. The mycelium is destroyed at lower temperatures than the spores, so that, at 90°, *A. griseus* survived only in the spore form, while the mycelium was destroyed; the mycelium of *A. scabies* seems to be destroyed at 80°, although the spores survive that temperature and are destroyed at 90°.

A temperature of 75° is sufficient to destroy the rennetic enzyme of these organisms. *A. griseus* milk cultures added to fresh sterilized milk and kept for 24-48 hours at 75° gave no action upon the milk. The temperature has an effect not only upon the rapidity of clotting and subsequent peptonization of the milk by the cultures but also upon the mode of action. Twenty tubes of

sterile milk were inoculated with *A. griseus* and 20 tubes with *A. exfoliatus*, and 10 tubes of each culture placed at 37° and at 25°. At 37°, all the *A. griseus* cultures formed a clot in 5-6 days and digestion began in 6 days and was nearly completed in 10 days. Of the 10 cultures of *A. exfoliatus*, 3 clotted in 9 days and all were clotted in 13 days; the digestion of the milk in these cultures began in 13 days and was not completed in 30 days. At 25°, of the 10 *A. griseus* milk cultures, 2 clotted in 6 days and all clotted in 10 days; peptonization of the clot began soon after this was formed and was completed in 14-15 days. *A. exfoliatus* produced no clot on the 10 tubes at 25° but hydrolysis of the milk became apparent in 25 days and most of the tubes cleared up in 30-35 days.

PRODUCTION OF ENZYMES

With the exception of the rennetic and proteolytic enzymes, as was shown elsewhere, no attempts were made to isolate the enzymes of the different actinomycetes. Most species produce readily diastatic, rennetic and proteolytic enzymes. Some produce a cellulase, as demonstrated by the cellulose plate method; invertase is produced only by a few species.

The production of tyrosinase has been pointed out in another connection in this paper. The hemolysis of the blood is no doubt accomplished by an hemolysin-like enzyme, which is produced by the most active proteolytic species. The proteolytic enzyme liquefies and splits gelatin readily; casein is readily hydrolyzed; the fact that coagulated blood serum is liquefied while coagulated egg-albumin, with very few exceptions, is not liquefied by many species would seem to indicate that the former is attacked by the proteolytic enzyme, while the latter is not. When milk cultures of a few species were filtered and filtrates precipitated by means of alcohol, preparations were obtained which possessed very active rennetic and proteolytic properties.

SUMMARY OF COMPARATIVE CULTURAL DATA

1. All the actinomycetes can be grown readily in artificial culture media, both synthetic and organic.
2. Some species show distinctive cultural differences when grown in artificial media soon after isolation from natural substrata and after they were kept in culture for a number of years. This is true only of some species and not of all of them.
3. Arabinose is not assimilated by most species. Dextrose, maltose, lactose, mannite, glycerin and starch are readily assimilated by most species to a greater or less extent, very few cultures producing only a scant growth on these sources of carbon; saccharose is assimilated readily by some species, although nearly all organisms made some growth on this source of carbon, particularly in agar media. Cellulose is readily assimilated only by some species. Inulin is readily assimilated by most species.

4. NaNO_3 is assimilated by all species in the presence of a favorable source of carbon. NaNO_3 is readily assimilated by most species but only in very low concentrations. Ammonium salts are assimilated readily only by very few species, but in the presence of favorable sources of carbon, such as dextrose, they are assimilated to some extent by many species. Urea and acetamide are assimilated only to a very small extent, and only few organisms make a very good growth on these sources of nitrogen. The proteins and amino acids form the best sources of nitrogen for most actinomycetes; creatinine is readily used, but not to such an extent as the proteins.

5. Most actinomycetes grow readily on milk. Very few produce any visible surface growth at 37° , but produce a good growth at 25° ; the milk is usually clotted and peptonized; few species hydrolyze the milk, without any previous clotting and some produce no visible action upon the milk; the hydrolysis and the lack of visible action upon the milk by some species is not absolute, since some of these cultures may at other times, particularly at favorable temperatures, clot and peptonize the milk. The reaction of the milk is usually changed to alkaline; no species renders the milk acid, while a few do not change the reaction of the milk.

6. Gelatin in distilled water forms a good medium for nearly all species. The gelatin is liquefied by nearly all species with different rapidity, with or without the production of a soluble brown pigment in the liquefied and often unliquefied portion.

7. Some species are characterized by the production of a brown pigment in gelatin as well as in other media containing proteins or amino acids; this pigment is not due to the action of tyrosinase only, since on tyrosin only a few species produced the pigment, while the latter was also produced in media not containing tyrosin.

8. Blood serum, blood agar and whole egg form good media for the cultivation of most species; liquefaction of the coagulated serum, hemolysis of the blood and the production of a soluble purple to black pigment on all the three media is characteristic of several species.

9. Potato and carrot can be readily used for the cultivation of most species, some of which produce a characteristic growth.

10. The optimum range of temperature for the growth of most actinomycetes is $30-37^\circ$. The lower the temperature, the slower is the growth of the organisms. Above 37° , the growth rapidly diminishes and at 45° only one organism made a scant growth. A temperature of 80° for 1 hour is sufficient to kill most species; only one form (*A. griseus*) survived that temperature and was killed when kept at 90° for 1 hour. The character of the growth and biochemical activities may vary somewhat with the different temperatures.

11. Most of the actinomycetes are very active proteolytically, splitting the proteins to amino acids and ammonia. In this respect they differ somewhat from some molds and some bacteria which produce a great deal of ammonia as a final product. The protein hydrolysis may stop at the amino acid (poly-

peptide) stage and very little of it may be reduced to ammonia, particularly in a short period of incubation; when the period of incubation is prolonged (30-60 days) very large quantities of ammonia accumulate.

12. Most actinomycetes reduce nitrates to nitrites, depending on the source of carbon; some species do that only to a very limited extent and with one or two sources of carbon, while others reduce nitrates readily with practically all sources of carbon. The nitrite production seems to be a step of the utilization of the nitrate nitrogen for at least some organisms; this may explain the absence of nitrites in certain cases, since the nitrite is assimilated by the organism as soon as formed. The organisms that reduce nitrates readily can use nitrites as the only source of nitrogen, when present in small amounts.

13. The following enzymes are produced by most actinomycetes species: rennet like, proteolytic and diastatic enzymes. Inulase and invertase are produced only by certain species, and tyrosinase by very few. The hemolysis of blood and the utilization of cellulose may also be brought about by the particular enzymes.

14. In summarizing the cultural and biochemical studies, the following media can be recommended for a starting point in studying and identifying the different species:

(a) Synthetic agar No. 1 and glucose agar (Krainsky's) as standard synthetic media; temperature of incubation 22-25°, period of incubation 7-15 days.

(b) Gelatin, 15 per cent, in distilled water; reaction unadjusted; temperature of incubation 16-18°; period of incubation 30 days.

(c) Skimmed milk; temperature of incubation 25° and 37°; observations to be made daily.

(d) Potato plugs at 25° for 7-15 days.

(e) Starch agar at 25° for 10-15 days, test for diastatic power.

(f) Nutrient agar, 25° for 7-15 days (optional).

(g) Tyrosin solution, 25° for 15-20 days (optional).

(h) Loeffler's blood serum, 37° for 7-15 days (optional).

VARIABILITY

The species of Actinomycetes are among the most variable groups of microorganisms; in the study of actinomycetes, as well as bacteria, not only the influence of the medium and previous substrata on which the particular species were grown, but the great variability, within rather wide limits, of the species or rather "species group" itself should be considered. When the same culture grown on different artificial culture media for a period of time, and often even for one short generation, is studied morphologically and physiologically, notable variations are observed, so that the untrained observers would be apt to take these as representing different forms. Often several strains of the same "species group," when compared on the same culture medium, might be taken

culturally and often morphologically as distinctly different species. Quite often one culture, on the same medium, under the same conditions of temperature and length of incubation, will show distinctive differences.

How shall then the limits of variability of a particular species be defined? The morphological characters alone would be far from sufficient. First of all, the substratum mycelium is not very characteristic and is quite uniform for many species; only the aerial mycelium shows the distinctive morphological characters. But the aerial mycelium may often not be produced at all by several species; others may not form any aerial mycelium on certain media, while forming it on others; still other species may lose the ability to form the aerial mycelium altogether on continued cultivation on artificial culture media. Then, the same culture may show slight differences in morphology on the same medium at different periods, or at one period on the different media. Even more striking than the morphological are the physiological variations. These depend chiefly on the substratum of the mother culture, temperature and length of incubation, amount and kind of inoculum (vegetative or aerial mycelium or spores). For example, a certain culture (*A. griseus*) may at one time clot the milk at 37° in 2 days and then peptonize it (dissolve the clot) in 5-6 days; at another time, the same culture, under the same conditions, will clot the milk only in 5-6 days, then peptonize it in 12-15 days; while at a third time, some tubes may not show any clot at all, and the milk is hydrolyzed (cleared up without any previous clot). Another culture (*A. exfoliatus*) may show persistently an ability to hydrolyze milk without forming any clot at 37°, then it may form a very good clot, followed by peptonization; when incubated on the same lot of milk from the same mother culture, it may show a clot followed by peptonization at 37°, while at 25° only hydrolysis may take place, at another time a clot is formed also at 25°. The rapidity of liquefaction of gelatin is also subject to a great variation. Still more intense is the variability, when such factors as the amount of split products obtained from a certain protein or carbohydrate are considered. All these factors come to show that ordinary bacteriological methods will not hold when applied to this group of microorganisms. The amount of variation cannot be fixed so easily and if it were done, it would be very arbitrary.

On closer intensive study of this group of organisms, we find that the variations, although often very striking, are mostly of a quantitative rather than qualitative nature. It was suggested above that these organisms should be classified in groups rather than as individual species. The characters, particularly the cultural and biochemical, should be studied repeatedly, under different conditions, then the data obtained compared, due allowances being made for the variability, and the culture will then be properly located. This idea was kept continuously in mind in the above studies; many of the data show rather a range, obtained from repeated cultures, than a single observation. If attempts were made to describe all the details of the cultures and to make new

species based on some variations from others, the 40 or so species described above could have been easily increased to several thousand, the task becoming more and more laborious and almost unnecessary. And, since it became almost impossible to carry on in continuous culture numerous strains, all of them were studied on several media, which allowed a better differentiation of their morphological and cultural differences. The great majority of the strains were then discarded and only the strains representing the "species groups" left. Repeated study and collective comparison of data have helped to bring forth some of the distinctive characters of these "species groups." An example of forming one can be found in the strains of *A. griseus* and *A. 218* which are as different from one another as any two strains of one "species group" (with the exception of the "*A. scabies*" group); descriptions of both of them are given so as to indicate the extent of variability allowed by the writer.

SAPROPHYTES AND PARASITES

Most of the species studied in this paper are saprophytic in nature. *A. scabies* is pathogenic to plants, while *A. madurae*, *A. hominis*, *A. bovis* and *A. asteroides* are supposedly animal pathogens. There is absolutely no difference between the pathogenic and saprophytic forms. Parasitism of a few species is not sufficient reason to have them separated in a different group and all attempts in that respect are only superficial in nature. The methods outlined for the study of this group are applied just as readily to both saprophytes and parasites. The only distinctive difference is found in the optimum incubation temperature: that of the animal pathogens is higher than that of saprophytes and plant pathogens, which we would naturally expect.

MORPHOLOGICAL STUDIES

The reasons have been presented above, why the morphological studies here will have to be only very limited, in view of the very detailed studies of Drechsler (13). Attention will be called here to only a few facts, chiefly the variability of the morphological characters depending on the culture medium. It is well to describe these organisms on one definite medium, but when several media are used, great variations are obtained. It is then very important to have the one medium used of exact chemical composition so that the work may be readily repeated. A few plates are given in the end of this paper to point out this variability and also to indicate some characteristic morphological features of the different species.

Three distinct morphological types are recognized:

- a. Whirl formation, as represented by only two species: *A. reticuli* and *A. reticulus-ruber*. This feature is very characteristic and cannot fail to help recognize the group, but it was observed only on the synthetic (saccharose) agar, and even on this medium, the second species has shown only limited whirl formation (plate 3).

b. Those species that do not form any spirals in the aerial mycelium, forming only straight branching hyphae. Also in this respect, we find great variability on the different media (plate 4).

c. Those species that form spirals. These comprise the majority of the actinomycetes, and distinctive differences are found in type and method of formation of spirals as well as in the variation on the culture medium. Some form wide, open spirals, while others form narrow spirals of a corkscrew type (plates 1 and 2). The division into dextrorse and sinistrorse types of spirals has already been pointed out by Drechsler (13).

KEY TO THE IDENTIFICATION OF THE SPECIES

(Based chiefly on biochemical characters)

A. Formation of a soluble pigment on all media containing protein substances:

I. Pigment deep brown (chromogenus types):

1. A brown pigment is produced on tyrosin agar:

- (a) Pigment dark brown; white to cream-colored growth on synthetic media; soluble brown pigment on synthetic media containing arabinose, dextrose or lactose.....*A. scabies*

- (b) Pigment faint brown; sulfur-yellow soluble pigment on creatinine solution; aerial mycelium on glucose agar is ochre to reddish ochre colored.

Actinomyces 205

2. Growth and aerial mycelium on synthetic agar green to dark-green; soluble brown pigment on synthetic media with most carbohydrates.

A. viridochromogenus

3. Deep brown to black pigment on synthetic agar:

- (a) Weakly growing organisms; orange-red growth on potato plug; no visible aerial mycelium on synthetic agar.....*A. purpeochromogenus*

- (b) Vigorously growing organisms; brown to black growth on potato plug; abundant cottony aerial mycelium on synthetic agar.

A. pheochromogenus

4. Usually no action on milk (37°), accompanied by the darkening of the milk; mouse-gray aerial mycelium on synthetic agar; ammonium salts used readily with different sources of carbon.....*A. aureus*

5. Brown pigment never produced on synthetic media:

- (a) Aerial mycelium on synthetic media has lavender shade...*A. lavendulae*

- (b) Aerial mycelium on synthetic agar is abundant, of a water green color.

Actinomyces 218

(c) Whirl formation in aerial mycelium on synthetic agar:

- (a') Growth colorless and aerial mycelium white.....*A. reticuli*

- (b') Growth pink, aerial mycelium rose colored; nitrate reduction very abundant; fewer whirls.....*A. reticulus-ruber*

- (d) Growth on synthetic agar sulfur-yellow, with yellow aerial mycelium; barnacle-like, greenish-yellow growth on potato plug.....*A. flavus*

- (e) Growth on synthetic agar red colored, aerial mycelium abundant, orange colored; aerial hyphae usually do not form spirals.....*A. ruber*

II. Soluble pigment on organic media faint brown, golden, yellow or blue:

1. Pigment blue, not always definite; soluble red turning blue pigment on synthetic agar.....*A. violaceus-ruber*

2. Pigment at first green on organic media and synthetic agar, property lost on continued cultivation, becoming brown on synthetic agar; aerial mycelium not produced on most media.....*A. verne*
3. Soluble pigment at first brown, property lost entirely on continued cultivation; growth and aerial mycelium on synthetic agar abundant, white.

A. albus

4. Soluble pigment yellowish green; growth on synthetic agar penetrating into the medium is pink.....*A. californicus*
5. Soluble pigment on organic media (gelatin and glucose broth) golden; sulfur-yellow growth on synthetic agar with yellow soluble pigment (last property lost on continued cultivation).....*A. 168*
6. Brown pigment produced only on certain protein media (usually gelatin and glucose broth, not nutrient agar):
 - (a) Growth on synthetic agar red to pink; no differentiated aerial mycelium or only scant white.....*A. bobili*
 - (b) Growth on synthetic agar colorless; aerial mycelium thin, rose-colored.
A. roseus
 - (c) Growth on carrot and potato rapidly spreading, enveloping the whole plug and destroying it rapidly, plug becoming colored deeply brown.
A. 96
 - (d) Red (vinaceous) soluble pigment on synthetic agar, often turning red-brown; white aerial mycelium.....*A. 161*
 - (e) Reaction of organic media always becoming acid; weak proteolytic action; very coarse aerial hyphae, without any spiral production.

Actinomyces 145

B. No soluble pigment produced on gelatin or other protein media:

I. Species strongly proteolytic; gelatin liquefied rapidly, milk clotted and peptonized rapidly.

1. Brown soluble pigment on synthetic agar; diastatic action very strong.

A. diastaticus

2. Rapid liquefaction of coagulated blood serum, strong hemolysis of blood (37°):
 - (a) Very poor utilization of glycerin as a source of energy; aerial mycelium on synthetic agar has a tea-green tinge.....*A. griseus*
 - (b) Colorless growth on synthetic agar with white aerial mycelium; pink growth on glucose agar with scant aerial mycelium; growth slow at 25°, rapid at 37°; pathogenic to man.....*A. madurae*
 - (c) Yellowish growth on the 3 synthetic agars; aerial mycelium on synthetic agar has an olive-green tinge (very similar to that of *A. griseus*); yellowish to orange, growth turning brown on potato plug; olive-colored growth on carrot; pathogenic to man.....*A. hominis*
3. Yellowish green growth on starch plate with pinkish aerial mycelium; citron-yellow growth on synthetic agar.....*A. citreus*
4. Greenish-yellow growth on synthetic agar, gray powdery aerial mycelium, greenish-yellow soluble pigment.....*A. 128*
5. Colorless growth on synthetic agar, white to grayish aerial mycelium, no spiral formation; thin reddish-brown growth on potato plug (purplish zone on plug); faint yellow pigment may develop on gelatin, blood and egg-media.

A. poolensis

6. Buff colored growth on glucose agar, violet-gray aerial mycelium; yellow growth on synthetic agar with light drab aerial mycelium; rapid destruction of potato plug.....*Actinomyces 206*
7. Proteolytic action somewhat weaker than previous members of group B I, although much stronger than the species included in B II.

- (a) Yellow growth on most synthetic and organic media, often turning black, sulfur-yellow aerial mycelium, which usually develops only on continued cultivation; pathogenic to animals.....*A. bovis*
- (b) Very scant colorless growth with scant white aerial mycelium on synthetic agar and on synthetic media containing NaNO_3 as a source of nitrogen; abundant brown growth with white aerial mycelium and soluble brown pigment on glucose agar; growth on potato plug greenish turning black.....*Actinomyces* 104

II. Proteolytic action weak:

1. Soluble pigment produced on synthetic agar:
 - (a) Pigment blue or blue-black.....*A. violaceus-caesari*
 - (b) Pigment brown to almost black on all synthetic media with NaNO_3 as a source of nitrogen.....*A. exfoliatus*
2. No soluble pigment on synthetic agar, although growth is colored:
 - (a) Growth turning black, diastatic action very strong:
 - (a') Growth on synthetic agar scant with abundant spirals in aerial mycelium, no invertase production.....*A. rutgersensis*
 - (b') No spirals on synthetic agar, characteristic green colored growth on protein-glycerin media.....*A. lipmanii*
 - (c') None or scant aerial mycelium on all media; growth abundant on synthetic agar (invertase positive); none or scant growth on blood agar and egg-media.....*A. halstedii*
 - (b) Growth orange colored on most synthetic and organic media:
 - (a') Aerial mycelium pink.....*A. fradii*
 - (b') White aerial mycelium may or may not be produced; proteolytic and diastatic action lacking almost entirely (this is the only species which does not liquefy gelatin and has no visible effect upon milk at 25° and 37°).....*A. asteroides*
 - (c) Growth yellowish on synthetic and glucose agars: pinkish cinnamon-colored on calcium malate agar; no growth on blood serum and egg media; none or only very scant and late aerial mycelium on most media.....*A. alboblavirus*
 - (d) Growth on synthetic media rose to red colored, aerial mycelium white, no visible action on milk.....*A. albosporus*

The writer is well aware of the several criticisms to which this key could be subjected. First of all, pigment production, which is never an absolutely reliable factor and which is subject to variations, is made use of in the major and minor subdivisions. Second, the major divisions of part B are based on quantitative differences in proteolytic action, which also is not always reliable. With all these criticisms in mind, the present key was decided on, since it presents a brief summary of the more important biochemical features of the different species which will help to separate them. It will no doubt be of help to those who will attempt to locate a certain culture. The key should of course be used only as a preliminary step in the identification; for further details, one should refer to the complete description. At least those media should be used which are recommended in the last paragraph of the summary of the cultural characters.

Attention is here called to the fact that the most of the cultural studies reported above were repeated several times at different laboratories by the writer and often also by others (associated with the writer, notably Mr. Curtis and Mr. Joffe of the New Jersey Agricultural Experiment Station).

TABLE 1

*The utilization of different carbon compounds by actinomycetes**

ORGANISM	CARBON SOURCE												
	Arabinose	Dextrose	Saccharose	Lactose	Maltose	Starch	Inulin	Cellulose†		Mannite	Glycerin	Acetate	Lactate
								I	II				
<i>A. violaceus-ruber</i>	1	5	2-3	5	4	5	2	0	3	3	3	1	—
<i>A. violaceus-caesari</i>	1	3	2	1	2	3	—	2	3	1	0	1	1
<i>A. aureus</i>	0	4	1	4	—	2	—	0	0	2	3	1	1
<i>A. scabies</i>	4	2	2	4	2	4	—	1	—	3	3	1	1
<i>A. viridochromogenus</i>	3	4	2	3	4	3	2	0	3	3	2	1	2
<i>Actinomyces</i> 205.....	0	1	1	2	4	3	2	2	0	1	1	1	0
<i>A. albus</i>	2	3	2	3	3	3	—	2	0	3	3	1	2
<i>A. exfoliatus</i>	4	4	5	4	3	4	2	0	1	3	3	1	1
<i>A. griseus</i>	3	4	1	3	4	4	1	0	0	3	2	1	0
<i>A. alboporeus</i>	0	3	2	2	1	3	—	1	0	4	1	1	1
<i>A. lipmanii</i>	0	3	3	3	—	1	—	3	—	3	3	—	—
<i>A. diastaticus</i>	4	4	1	4	3	3	2	2	0	4	4	0	1
<i>A. bobili</i>	0	2	1	3	2	3	—	1	0	0	3	2	1
<i>A. poolensis</i>	0	1	1	2	1	3	—	3	0	1	4	0	0
<i>A. fradii</i>	3	4	1	3	2	4	2	0	0	3	3	1	1
<i>Actinomyces</i> 128.....	0	2	1	2	3	5	—	3	0	1	1	1	1
<i>A. roseus</i>	1	3	2	0	—	4	—	0	—	1	2	—	—
<i>A. verne</i>	0	1	1	2	2	3	—	1	1	3	1	—	1
<i>A. reticulatus-ruber</i>	0	4	1	2	4	1	2	1	1	0	3	2	1
<i>Actinomyces</i> 168.....	0	5	2	4	3	2	—	2	1	5	4	1	2
<i>A. ruber</i>	1	3	2	3	4	4	3	2	2	3	3	2	0
<i>A. asteroides</i>	0	4	3	0	3	4	—	0	1	0	2	1	2
<i>Actinomyces</i> 104.....	0	1	1	1	2	0	—	1	1	1	1	1	1

* NaNO_3 (2 gm. per liter) used as a source of nitrogen. All carbon compounds were used in a 3 per cent concentration.

† I, designates filter paper; II, reprecipitated cellulose.

‡ Figures enclosed in parentheses designate the utilization of the malate in the form of the ammonium salt, as the only source of nitrogen and carbon.

TABLE 2
The utilization of different nitrogen compounds by actinomycetes*

ORGANISM	NITROGEN SOURCE																	
	Fibrin	Casein	Egg-Albumin	Peptone	Creatinine	Glycocoll	Leucin	Tyrosin	Asparagin	Urea		Acetamide	NaNO ₂	NaNO ₃	(NH ₄) ₂ SO ₄ †		(NH ₄) ₂ CO ₃ †	
										g	d				g	d	g	d
<i>A. violaceus-ruber</i>	{ 3† 3	3 4	2 2	3 4	3 —	3 4	3 3	2 —	3 4	1 2	5 —	2 1	1 1	3 3	0 0	1 —	0 0	4 —
<i>A. griseus</i>	{ 4 4	5 5	4 4	5 5	— 3	4 3	3 3	— 3	3 1	— 1	— —	1 1	1 2	1 1	0 1	— —	0 0	— —
<i>A. aureus</i>	{ 3 4	4 5	4 5	4 5	3 —	5 5	4 5	3 —	4 4	1 1	4 —	2 2	3 5	3 3	1 5	2 —	1 2	4 —
<i>A. bobili</i>	{ 2 3	3 4	1 3	2 4	— —	2 3	2 —	— —	1 1	1 —	1 —	1 2	1 —	0 0	0 0	— —	0 0	2 2
<i>A. scabies</i>	{ 3 5	3 4	2 3	3 4	3 —	2 3	2 3	2 —	2 2	1 2	2 2	1 —	1 2	1 3	0 0	— —	0 0	3 —
<i>A. albus</i>	{ 3 3	2 3	3 4	2 4	— 4	3 3	3 —	2 —	1 —	— 1	— —	1 1	1 5	1 1	0 1	— —	0 0	— —
<i>A. viridochromogenus</i> ...	{ 3 3	4 5	3 —	4 4	3 —	3 5	3 3	3 —	2 2	3 —	4 —	1 1	2 2	1 3	0 0	1 —	0 0	3 —
<i>A. verne</i>	{ 2 2	2 3	2 3	3 —	— 3	3 3	3 —	2 3	1 1	1 —	— —	1 —	1 —	1 1	0 0	0 —	0 0	3 —
<i>A. bovis</i>	{ 2 3	3 4	3 4	2 2	— 2	2 2	2 —	2 3	1 1	2 —	1 —	1 1	1 —	1 —	0 0	1-2 0	0 0	2 —
<i>A. asteroides</i>	{ 1 1	1 1	3 3	2 2	— 2	2 2	— 2	— 1	1 1	4 —	— —	1 1	1 —	0 —	0 —	2 —	0 0	3 —
<i>A. reticuli</i>	{ 4 4	— —	1 —	4 —	— —	3 —	3 —	— —	3 —	0 —	1 —	0 —	1 —	1 —	0 —	0 —	0 —	1 —
<i>Actinomyces 205</i>	{ 3 5	3 4	2 3	3 4	3 —	3 5	3 4	3 —	3 5	0 3	— —	1 2	2 2	1 1	2 2	— —	0 1	— —

* Organic nitrogenous substances were used in concentrations of 0.5 per cent (creatinine and tyrosin only 0.1 per cent); nitrite, nitrate and ammonium salts 0.2 per cent.

Three per cent of glycerin was used as a source of energy for all nitrogen compounds unless otherwise designated.

† Column marked "g" designates glycerin and "d" designates dextrose (3 per cent) as source of energy.

‡ 0, designates no growth; 1, faint; 2, fair; 3, good; 4, very good; 5, excellent; —, not tested. The figures in the upper row were obtained on incubating the cultures for 15-18 days (*A. bovis* and *A. asteroides* for 30 days) at 25°; those in the lower row were obtained for the cultures incubated 30-35 days (*A. bovis* and *A. asteroides* 60 days).

TABLE 3
The growth of actinomycetes on milk

ORGANISM	INCUBATION AT 37°-DAYS						INCUBATION AT 25°-DAYS									
	Coagulation	Peptonization			Hydrolysis	No action	Browning	Reaction	Coagulation	Peptonization			Hydrolysis	No action	Browning	Reaction
		Start	End*	Rapid-ly						Start	End*	Rapid-ly				
<i>A. albobanus</i>	-	-	-	-	10-12	-	-	++	-	10-12	20+	-	20+	-	-	7.5
<i>A. albosporus</i>	-	-	-	-	-	+	0	+	-	-	-	-	20	-	-	7.7
<i>A. albus</i>	-	-	-	-	20	+	+	+	-	-	-	-	20-25	+	-	8.0
<i>A. asteroides</i>	-	-	-	-	-	+	0	-	-	-	-	-	-	-	+	6.2
<i>A. aureus</i>	-	-	-	-	-	+	0	-	-	-	-	-	-	-	+	7.3
<i>A. bobili</i>	-	-	-	-	15-18	-	+	++	-	-	-	-	15	-	+	8.0
<i>A. bonis</i>	10-12	40	+	+	-	+	++	++	10-12	20+	+	+	-	-	-	8.2
<i>A. californicus</i>	15-15	30+	+	+	-	+	++	++	5-6	15-20	+	++	-	-	-	8.0
<i>A. citreus</i>	9-10	9-10	20	+	-	+	++	++	5-6	20	+	++	-	-	-	7.0
<i>A. chromogenus</i> 205.....	5-7	7	18-25	+	-	+	++	++	4	20	+	++	-	-	+	8.0
<i>A. diastolicus</i>	6-7	7-8	50+	+	-	+	++	++	5-6	50+	+	++	-	-	+	5.4
<i>A. 161</i>	-	-	-	-	8-10	-	++	++	-	-	-	-	-	-	-	6.4
<i>A. exfoliatus</i>	-	-	-	-	-	-	++	++	5	20	-	-	-	-	-	7.8
<i>A. 128</i>	3-6	3-6	15-30	+	-	-	+	+	-	-	-	-	15	-	-	7.8
<i>A. flavus</i>	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	5.4
<i>A. fradii</i>	10-12	10-12	20	+	+	-	++	++	-	-	-	-	20+	-	-	7.8
<i>A. 96</i>	10-20	10-20	30	+	-	-	++	++	10	20	+	++	-	-	+	8.3
<i>A. griseus</i>	4-5	5	10	+	-	-	++	++	3-5	20	+	++	-	-	-	8.0
<i>A. 218</i>	-	-	-	-	15-20	-	++	++	-	-	-	-	20	-	+	7.6
<i>A. halstedii</i>	10	10	50+	+	-	-	++	++	8-10	30+	+	+	10	-	-	8.2
<i>A. hominis</i>	5-6	5-6	20	+	-	-	++	++	-	-	-	-	-	-	-	7.8
<i>A. laevendulae</i>	-	-	-	-	20-30	-	++	++	-	-	-	-	3-4	-	-	7.8
<i>A. lipmanii</i>	8-9	9-10	30	+	-	-	++	++	Soft	-	-	-	10-12	-	-	7.8

TABLE 4
Comparative cultures of actinomycetes on gelatin and liquid media

ORGANISM	GELATIN 15 PER CENT		GLUCOSE BROTH		SYNTHETIC SOLUTION		GLYCERIN SYNTHETIC SOLUTION	
	Pigment	Liquefaction*	Growth	Pigment	Growth†	Nitritest‡	Growth	Nitrites
<i>A. albolavus</i>	None	Rapid	Colonies	None	Colonies	3	Flakes	T
<i>A. alboboreus</i>	None	Rapid	Ring	None	Flakes	2	Flakes and colonies	2
<i>A. albus</i>	Brownish	Medium	Ring	None	Colonies	1-3	Flakes	1
<i>A. asterioides</i>	None	None	Pellicle	None	Flakes	2-4	Flakes	2
<i>A. aureus</i>	Brown	Medium	Ring	Brown	Flakes and colonies	0-1	Flakes	1
<i>A. bobili</i>	Brown	Rapid	Flakes	Brown	Colonies	3	Colonies and flakes	T
<i>A. bovis</i>	None	Medium	Ring	None	0	4	Flakes	4
<i>A. californicus</i>	None	Medium	Pellicle	None	Flakes	5	Pellicle	O
<i>A. citreus</i>	None	Medium	Ring	None	Flakes	0	Flakes and pellicle	T
<i>A. chromogenus</i> 205.....	Brown	Slow	Ring	Brown	Flakes	1	Pellicle and flakes	T
<i>A. diastaticus</i>	None	Rapid	Colonies	None	Flakes	—	Pellicle	T
<i>A. 161</i>	None	Slow	Ring or pellicle	Brown	Ring	0-2	Pellicle and flakes	T
<i>A. exfoliatus</i>	None	Slow	Colonies	None	Colonies	3	Colonies	T
<i>A. 128</i>	None	Rapid	Pellicle	None	Colonies	0-1	Flakes	1
<i>A. flavus</i>	Brown	Rapid	Colonies	None	Colonies	1	Colonies and pellicle	T
<i>A. fradii</i>	None	Rapid	Ring	None	Colonies	3	Pellicle and flakes	1
<i>A. 96</i>	Yellow	Medium	Ring	Brown	Flakes	0	Flakes	2
<i>A. griseus</i>	None	Rapid	Pellicle	Brown	Flakes	1	Flakes	T
<i>A. 218</i>	Brown	Medium	Ring	Brown	Flakes	2	Pellicle	3

TABLE 5
Summary of comparative cultures of actinomycetes on agar media

ORGANISM	SYNTHETIC AGAR			CALCIUM MALATE AGAR		GLUCOSE AGAR		NUTRIENT AGAR			STARCH AGAR	
	Spirals	Growth*	Aerial mycelium*†	Growth	Aerial mycelium	Growth	Aerial mycelium	Growth	Aerial mycelium	Soluble pigment	Growth	Aerial mycelium
<i>A. violaceus-ruber</i> ...	Numerous	Red-blue	Gray	Red	Gray	Brick-red	Gray	Colorless-red	Gray	None-reddish brown	Pink	Gray
<i>A. violaceus-caesari</i> ..	Numerous	Gray-bluish	White	Blue	White	Red	White	Gray	None	None	Bluish	Gray
<i>A. viridochromogenus</i>	Numerous	Dark-green	White-green	Dark-green	White-bluish	Gray-black	White-bluish	Gray	White	Brown	Yellowish	Greenish
<i>A. scabies</i>	None or few	Gray	White	Yellowish	Gray	Gray	None	White	None	Brown	White	White
<i>A. purpeochromogenus</i>	None or few	Brown	Black	Black	None	Brown	Dark-brown	Gray-Crown	None	Brown	Brown	None
<i>A. phaeochromogenus</i> ..	Numerous	Brown	White	Brownish	White	Brown	White	Gray	None	Brown	Brown	White
<i>Actinomyces</i> 205....	Numerous	White	Gray	White	Gray	Brown	White-gray	Brown	White	Brown	White	Gray
<i>A. aureus</i>	Numerous	White	Dark-green	White	Brown	Light-orange	Light-drab	Gray	None	Brown	White	Buff
<i>A. lavendulae</i>	Numerous	White	Lavender	White	Lavender	Yellowish	White-lavender	Gray	None	Brown	White	Lavender
<i>A. bobili</i>	None or few	Red	None-scant white	Cinnamon	None	Red	None	Gray-brownish	None	None	Pink	Scant white
<i>A. roseus</i>	Numerous	White	Vinaceous	White	Rose	White	Pink	White-yellowish	None	None	White	White

<i>A. diastaticus</i>	Few or none	Gray	Drab	Brown	Gray	Yellowish	None	White	White	None	White	None
<i>A. rutgersensis</i>	Numerous	Brown	Gray	White	Gray	Brownish	White	White	Gray	None	Gray	Gray
<i>A. lipmanii</i>	None	Brown	Gray	Brown	Gray	Yellow	None	Yellow	White-dark	None	White	None
<i>A. griseus</i>	None or few	White	Greenish	Green-yellow	Greenish	White	White	White	White	None	White	Gray
<i>A. 218</i>	None or few	White	Greenish	White	Greenish	Yellowish	Buff	Brown	White	Brown	White	White
<i>A. californicus</i>	Numerous	Pink	Gray	White	Gray	Pink	Gray	White	Pink	None	Pink	Gray
<i>A. albus</i>	None	Gray	White	Gray	Gray	Gray	Gray	White	White	None	White	None
<i>A. fredii</i>	None	White-orange	Pink	Orange	Pink	Buff	Pink	Orange	White	None	White	Pink
<i>A. exfoliatus</i>	None or few	Brown	White	White	White	Brownish	White	White	Brownish	None	Brownish	Gray
<i>A. reticulatus</i>	None (whirls)	Yellowish	White	White	Yellow	Brownish	Yellow	Brownish	Brownish	Brown	Brownish	Lavender
<i>A. reticulatus-ruber</i>	None or few (whirls)	Pink	Rose to pink	Red	Rose-pink	Red	Pink	Red	Pinkish	Brown	Pinkish	Lavender
<i>A. citreus</i>	Few	Yellow	Yellow	Yellow	Gray	Yellow	White	White	Yellow	None	Yellow	Pinkish
<i>A. alboflavus</i>	None	Yellowish	White-none	Pinkish	White (late)	Yellow	None	White	Yellowish	None	Yellowish	None
<i>A. verne</i>	None	Brownish	None	Avellaneous	Scant white	Gray	None	Gray	Brownish	None	Brownish	None
<i>A. albosporus</i>	None	Pink	White	Rose	White	Red	White	White	Reddish	None	Reddish	None
<i>A. hakstadii</i>	Few	Gray-brown	Gray	Dark	Gray	Dark-brown	None	White	Brown-white	None	Brown	None
<i>A. flavus</i>	None	Yellow	None	Yellow	White	Yellow	Scant white	Gray	White	Brownish	White	White
<i>A. poolensis</i>	None	White	White	White	Gray	Brown	None	Yellowish	White	None	White	White

TABLE 3—Continued

ORGANISM	SYNTHETIC AGAR			CALCIUM MALATE AGAR		GLUCOSE AGAR		NUTRIENT AGAR			STARCH AGAR	
	Spirals	Growth*	Aerial mycelium	Growth	Aerial mycelium	Growth	Aerial mycelium	Growth	Aerial mycelium	Soluble pigment	Growth	Aerial mycelium
<i>A. ruber</i>	None	Red	Orange	Orange	Yellow	Red	White-pinkish Gray	Olive green	Gray	Brown	Red	Pink
<i>A. 96</i>	None	White	Gray	Brownish	Gray	White to dark Yellow	Gray	Brownish	White	Brown	Brown	Gray
<i>A. 128</i>	None	Yellow	Gray	Yellow	Gray	Yellow	Gray	Yellow	Gray	None	Greenish-yellow	Gray
<i>A. 161</i>	Numerous	White-yellow	White	White-pink	White	White to brown	White	White	White	None	White	White
<i>A. 168</i>	Numerous	Yellow	Gray	White	Gray	White	Gray	White	White	None	White	Gray
<i>A. 206</i>	None	Yellow	Gray	Buff	Violet-gray	Buff	Violet-gray	White	None	None	Yellowish	White
<i>A. madurae</i>	None or few	White	White	White	Gray	Pinkish	White	White	White	None	White	None
<i>A. hominis</i>	None or few	Yellowish	Greenish	Yellowish	Greenish	Yellowish	White	Yellowish	White	None	White	None
<i>A. bovis</i>	Few	Yellow	Yellow	Brownish	None	Yellowish-dark	Yellow	Brown	Yellow	None	Yellow	None
<i>A. asteroides</i>	None	Orange	None	Orange	White	Orange	None	Yellow	White	None	Orange	White
<i>A. 104</i>	Numerous	White	Gray	Brownish	None	White	White	White	None	None	White	Gray
<i>A. 145</i>	None	White	Gray	White	None	White	Gray	White	Gray	None	White	Gray

* Only color of growth is reported under the column of growth.

† Only color is reported. For detailed description of color of growth and aerial mycelium see complete records.

TABLE 6
Summary of biochemical activities of actinomycetes

ORGANISM	INVERTASE PRODUC- TION	DIASTATIC ACTION		PROTE- OLYTIC ACTION	CHANGE IN REACTION
		Tube*	Plate†		
<i>A. alboflavus</i>	+	3	...	2-3	Variable
<i>A. albosporeus</i>	+	24	2-4	1-3	Little change
<i>A. albus</i>	-	14	3-4	1-3	Variable
<i>A. asteroides</i>	-	0	0	1	Acid
<i>A. aureus</i>	+	14	2	1	Variable
<i>A. bobili</i>	+	16	3-4	3-4	Unchanged
<i>A. bovis</i>	-	2	...	2	Alkaline
<i>A. californicus</i>	+	..	2-3	2	...
<i>A. chromogenus 205</i>	+	14	3	1-2	Variable
<i>A. citreus</i>	+	2	...	3	Alkaline
<i>A. diastaticus</i>	-	31	5	2-3	Alkaline
<i>A. 161</i>	+	..	3-4	3	Alkaline
<i>A. exfoliatus</i>	+	6	2-4	1-2	Variable
<i>A. 128</i>	-	16	1-3	3-4	Alkaline
<i>A. flavus</i>	+	15	2-3	2-3	Acid
<i>A. fragii</i>	-	17	3-4	3	Alkaline
<i>A. 96</i>	-	2	...	3	Alkaline
<i>A. griseus</i>	-	23	3-4	5	Alkaline
<i>A. 218</i>	-	3	...	3-4	Alkaline
<i>A. halstedii</i>	+	4	...	1-2	Little change
<i>A. hominis</i>	-	3	...	3-4	Variable
<i>A. lavenaulae</i>	-	3	...	1-2	Usually acid
<i>A. lipmanii</i>	+	24	4	3	Alkaline
<i>A. 168</i>	-	34	3-4	2-3	Alkaline
<i>A. madurae</i>	-	3	...	3-5	Alkaline
<i>A. pheochromogenus</i>	-	2	...	1-2	Alkaline
<i>A. poolensis</i>	-	5	2	3-5	Alkaline
<i>A. purpeochromogenus</i>	None— traces	1	...	1-2	Alkaline
<i>A. reticuli</i>	+	..	1-2	1-4	Acid
<i>A. reticulus-ruber</i>	+	12	1-2	1-2	Variable (acid)
<i>A. roseus</i>	-	3	...	3	Alkaline
<i>A. ruber</i>	+	14	2-3	2-3	Variable
<i>A. rugosensis</i>	-	22	5	3-4	Alkaline
<i>A. scabies</i>	+	0	0-1‡	1-2	Alkaline
<i>A. verne</i>	+	14	4	4	Little change
<i>A. violaceus-caesari</i>	-	15	...	2	Alkaline
<i>A. violaceus-ruber</i>	+	12	...	3	Alkaline
<i>A. viridochromogenus</i>	-	2	12	2	Variable
<i>A. 104</i>	-	8	1-3	2	Variable
<i>A. 145</i>	-	2	...	1-2	Acid

* Tube = Difference in the height of starch in the control tube and the tube in which the organism was grown, in millimeters.

† Plate = Width of clear zone around the colony, in millimeters. Period of incubation in both instances 12-15 days at 25°C. . . . designates "not tested."

‡ The starch is hydrolyzed chiefly to the dextrin stage.

TABLE 7
Comparative cultures of actinomycetes upon different media

ORGANISM	BLOOD AGAR			BLOOD SERUM			EGG-MEDIA			POTATO FLUG			CARROT FLUG		
	Growth	Soluble pigment	Hemolysis*	Growth	Soluble pigment	Liquefaction	Growth	Aerial mycelium	Soluble pigment	Growth	Aerial mycelium	Color	Growth	Aerial mycelium	Color
<i>A. alboblavus</i>	—	—	—	None	—	0	None	—	—	White	White	White	White	White	None
<i>A. albosporus</i>	—	—	—	Pink	None	0	Gray	None	—	Gray	None	None	White	None	None
<i>A. albus</i>	Greenish	None	0	White	None	0	White	White	Purplish	Red-brown	None	None	Pink	Green	None
<i>A. asteroides</i>	Brown	None	0	White	None	0	Yellow	White	None	Yellow	None	None	Orange	White	Brown
<i>A. aureus</i>	Brown	Dark zone	0	Gray	Dark zone	0	Brown	None	Purple	Brown	Gray	Black	None	—	—
<i>A. bovis</i>	Brownish	None	2	Gray	Yellow	3	White	White	None	Yellow	Yellow	Brown	White	Yellow	Dark
<i>A. bobillii</i>	—	—	—	Gray	Brown	0	Brown	None	Purple	Yellow-red	Scant white	Black	White	None	None
<i>A. californicus</i>	Gray	None	0	White	Brownish	1	White	Buff	Purplish	Brownish	White-buff	Brown	Gray	—	—
<i>A. chromogenus</i> 205.....	—	—	—	Brown	Dark zone	0	Gray	Gray	Black zone	Black	None	Black	Gray	White	Brown
<i>A. citreus</i>	Gray	None	1	White	None	0	Red	White	Black	Red	Pink	None	Red	None	None
<i>A. diastaticus</i> ...	Brown	Brownish	2	Gray	None	1	Yellow	None	Purple	Brownish	None	Brown	Brown	Gray	Brown
<i>A. 161</i>	—	—	—	Gray	None	0	Yellow	White	None	Yellow	Gray	Black	Yellow	Purple	Brown
<i>A. eufolatus</i>	—	—	—	White	None	0	Orange	White	None	Orange	White	None	Orange	White	None
<i>A. 128</i>	Green	White	4	Gray	White	4	White	White	None	Black	Green	Brown	Gray	Orange	Brown
<i>A. flavus</i>	—	—	—	White	Brown	0	White	None	None	White	White	Black	Green	None	None
<i>A. fradii</i>	Red	None	0	Orange	None	0	White	None	None	White	White	Purple	White	Gray	None
<i>A. 96</i>	—	—	—	None	—	—	Red	Rose	Dark zone	Green	Red	Black	Brown	None	None
<i>A. griseus</i>	Greenish	None	5	Gray	None	5	White	None	None	Yellowish	Greenish	Brown	None	Gray	None
<i>A. 218</i>	—	—	—	Gray	Brown	1	White	Buff	Purplish	Brownish	White-buff	Brown	Gray	—	—
<i>A. halstedii</i>	—	—	—	None	—	—	Gray	None	None	Gray	None	None	White	None	None
<i>A. hominis</i>	Gray	None	3	White	None	4	Yellow	None	None	Yellow	White	Brown	Olive	Green	None

<i>A. lavenderae</i>	—	—	—	Gray	0	White	None	None	White-yellow	None	Black	Brown	White	None
<i>A. lipmanii</i>	Gray	None	None	White	2	—	—	—	White	Gray	Purple	Gray	Gray	None
<i>A. 168</i>	Brownish	White	None	White	1	White	White	Pink	White	White	Purple	Brown	White	None
<i>A. madurae</i>	Brown	None	None	Yellowish	5	Yellow	White	None	Yellow	Gray	Brown	White	None	None
<i>A. phaeochromogenus</i>	Brown	None-dark	None-dark	Brown	1	Brown	White	Brown	Brown	White	Black	Brown	White	Black
<i>A. poolensis</i>	Greenish	None-brown	None-brown	Gray	1	Yellow	White	Purple	Green-olive	White	Dark	Yellow	None	None
<i>A. purpeochromogenus</i>	—	—	—	None	—	Gray	None	None	Orange	None	None	None	—	—
<i>A. reticulatus</i>	—	—	—	Gray	0	Yellow	None	None	Gray	None	None	White	None	None
<i>A. reticulatus-ruber</i>	—	—	—	Gray	0	Gray	Gray	Purple	Gray	Gray	Black	Yellow	None	None
<i>A. roseus</i>	—	—	—	Gray	0	Yellow	None	None	Brownish	None	Brown	Brown	None	None
<i>A. ruber</i>	Green	Dark gray	Dark gray	Yellow	0	Brown	None	Brown	Reddish-brown	None	Purple	Brown	Yellow	None
<i>A. rugosus</i>	—	—	—	Gray	0	Gray	White	Purple	Gray-brown	White	None	White	None	None
<i>A. scabies</i>	Gray	Dark brown	Dark brown	Brownish	0	Brown	None	Black zone	Black	None	None	None	—	—
<i>A. verne</i>	—	—	—	White	2	None	—	—	White	White	White	White	White	None
<i>A. violaceus-caesari</i>	—	—	—	Gray	0	Gray	None	None	Yellowish	None	None	None	—	—
<i>A. violaceus-ruber</i>	Red	Faint brown	Faint brown	Red	0	Brown	White	Blue	Brownish	White-gray	Blue	Brown	Gray	Pink
<i>A. viridochromogenus</i>	Brown	Dark brown	Dark brown	Brownish	0	Brown	White	Dark brown	Gray	White	Black	Gray	White	Brown
<i>A. 104</i>	—	—	—	Gray	1	White	None	None	Green	None	Black	Brown	None	Brown
<i>A. 145</i>	—	—	—	White	0	Brown	None	Purple	Gray	White	Black	White	None	Brown
<i>A. 206</i>	—	—	—	White	3	White	White	Pink	White	White	Brown	Brown	White	Brown

* Hemolysis at 37°: 1 = very narrow clear zone in 15 days at 37°; 5 = clear wide zone (2 mm. and more) in 10-15 days; 2, 3, 4 fall between, as relative amounts of hemolysis.

† 0 = no liquefaction at 37°; 1 = growth sinking into serum in 15 days at 37°; 5 = nearly all the slant liquefied in 10-15 days; 2, 3, 4 designate the relative amounts of liquefaction.

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PLATE 1

- FIG. 1. *A. violaceus-ruber* grown on dextrose agar.
FIG. 2. *A. violaceus-caesari* grown on dextrose agar.
FIG. 3. *A. violaceus-caesari* grown on calcium malate agar.
FIG. 4. *A. pheochromogenus* grown on dextrose agar.
FIG. 5. *A. pheochromogenus* grown on calcium malate agar.
FIG. 6. *A. purpureochromogenus* grown on starch agar.
FIG. 7. *A. aureus* grown on dextrose agar.

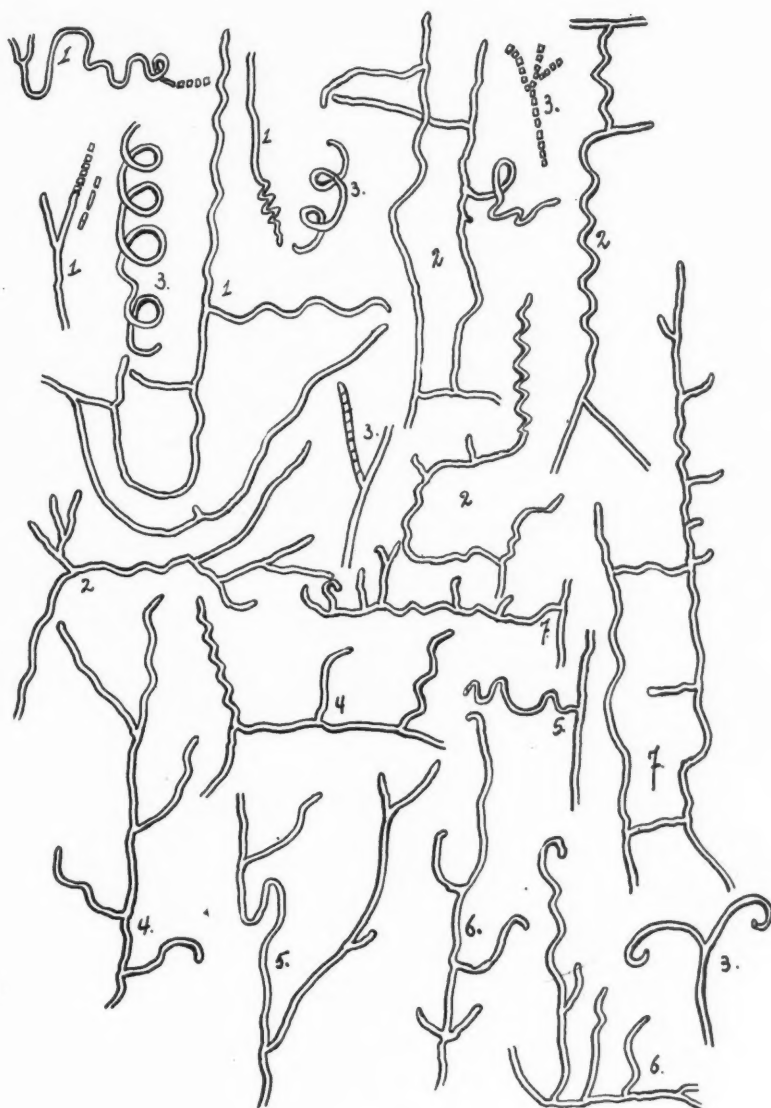


PLATE 2

- FIG. 1. *A. alboflavus* grown on calcium malate agar.
FIG. 2-3. *A. citreus* grown on dextrose agar.
FIG. 4. *A. exfoliatus* grown on dextrose agar.
FIG. 5. *A. californicus* grown on starch agar.
FIG. 6. *A. bobili* grown on starch agar.
FIG. 7. *A. ruber* grown on calcium malate agar.
FIG. 8. *Actinomyces* 218 grown on calcium malate agar.
FIG. 9. *A. bovis* grown on dextrose agar.

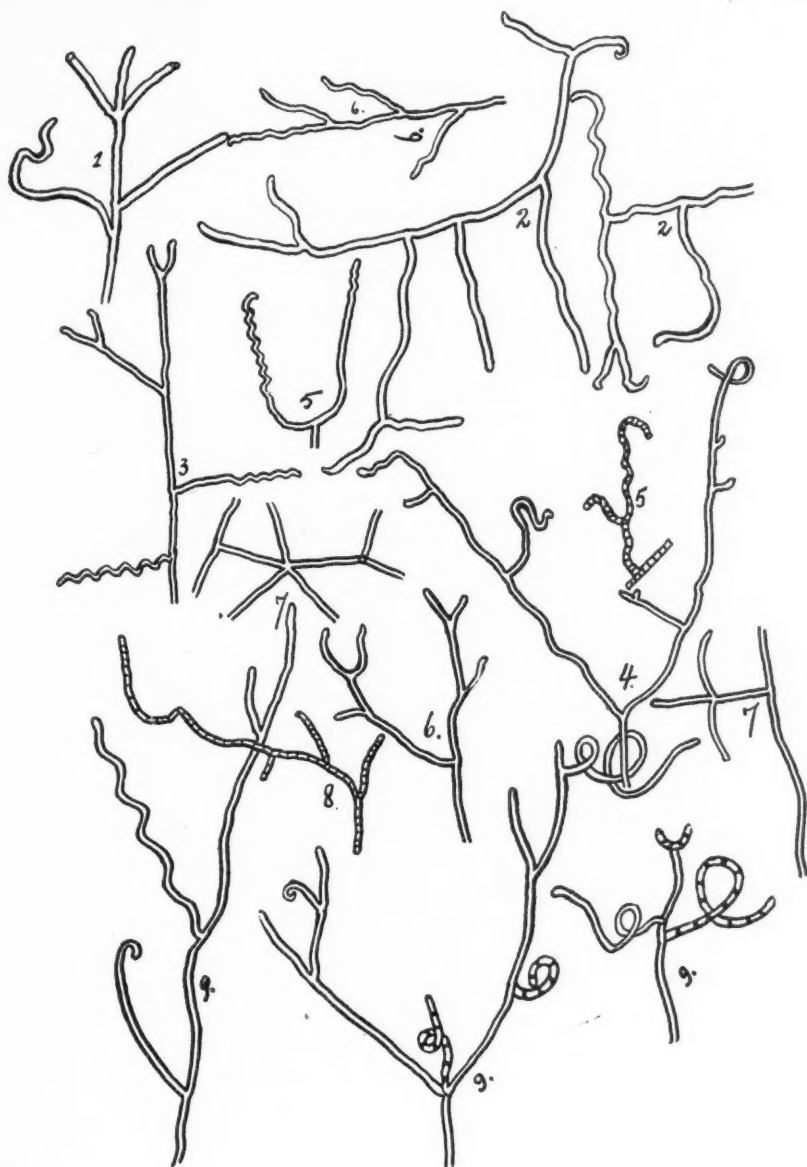


PLATE 3

- FIG. 1. *A. reticulatus-ruber* grown on dextrose agar.
FIG. 2-3. *A. reticuli* grown on dextrose agar.
FIG. 4. *A. reticuli* grown on synthetic agar.
FIG. 5. *A. reticuli* grow on glycerin-synthetic agar.

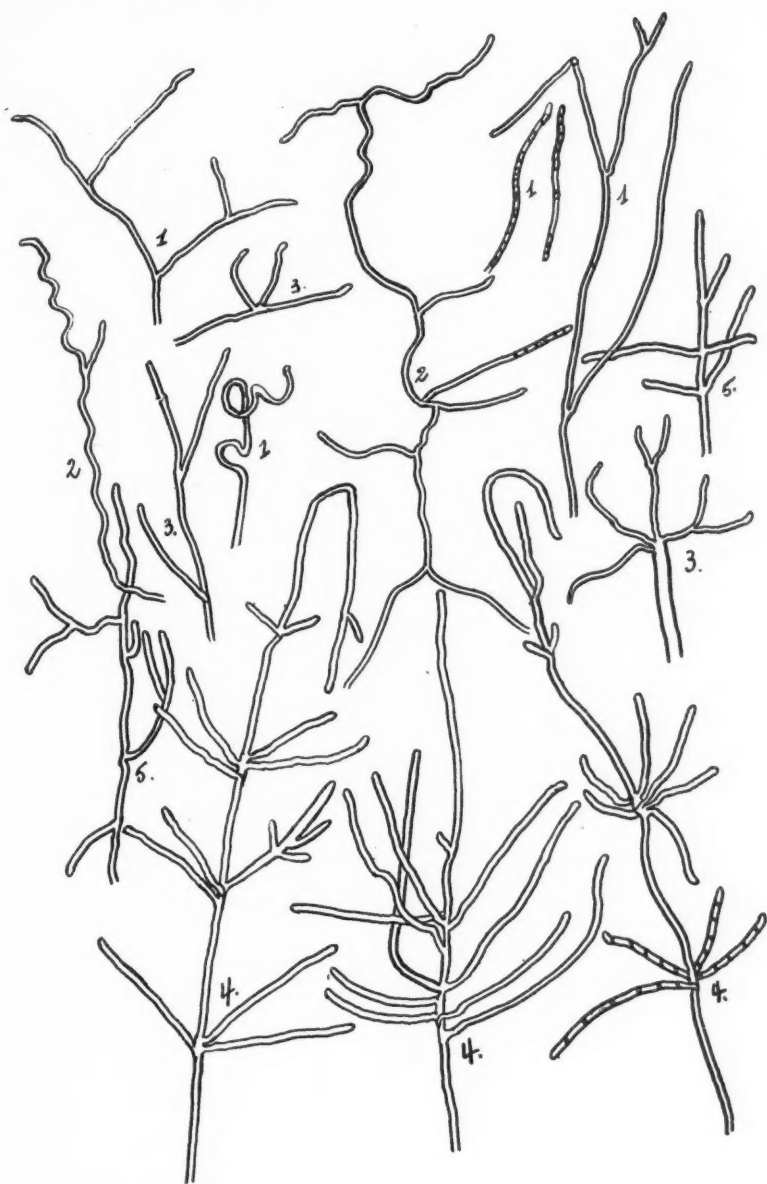


PLATE 4

- FIG. 1. *A. flavus* grown on starch agar.
FIG. 2. *A. flavus* grown on calcium-malate agar.
FIG. 3. *A. roseus* grown on starch agar.
FIG. 4. *A. roseus* grown on dextrose agar.
FIG. 5. *A. verne* grown on starch agar.
FIG. 6. *A. lavendulae* grown on dextrose agar.
FIG. 7. *A. flavovirens* grown on calcium malate agar.
FIG. 8. *Actinomyces* 145 grown on dextrose agar.
FIG. 9. *Actinomyces* 145 grown on calcium malate agar.
FIG. 10. *A. aureus* grown on glycerin synthetic agar.

